Brain

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Papers reporting experiments on patients or healthy volunteers must record the fact that the subjects' consent was obtained according to the declaration of Helsinki [*Br. Med. J* (1964) 2 177]. Care must be taken to preserve the anonymity of patients, in conformity with the statement of the Vancouver Group [*Br. Med. J.* (1991) **302** 1194] Consent must also be recorded when photographs of patients are shown or other details are given which could lead to the identification of the individuals Experiments on animals must conform to accepted ethical standards.

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BRAIN, SKELETAL MUSCLE AND PLATELET HOMOGENATE MITOCHONDRIAL FUNCTION IN PARKINSON'S DISEASE

by V. M. MANN,[1] J. M. COOPER,[1] D. KRIGE,[1] S. E. DANIEL,[2] A. H. V. SCHAPIRA[1,3] *and* C. D. MARSDEN[3]

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SUMMARY

The recent discovery of mitochondrial complex I deficiency in the substantia nigra of patients with idiopathic Parkinson's disease has provided new understanding into the possible mechanisms that may underlie this neurodegenerative disorder. The biochemical defect is identical to that induced in humans, primates and mice exposed to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. We have studied mitochondrial respiratory chain function in various brain regions, in skeletal muscle and in blood platelets from patients with idiopathic Parkinson's disease and from matched controls. We provide evidence suggesting that the complex I deficiency in Parkinson's disease is limited to the brain and that this defect is specific for the substantia nigra. The tissue specificity of the complex I deficiency in Parkinson's disease and its localization to the substantia nigra support the proposition that complex I deficiency may be directly involved in the cause of dopaminergic cell death in Parkinson's disease. An understanding of the molecular basis of this biochemical defect will provide valuable insight into the cause of Parkinson's disease. Our finding of normal mitochondrial function in platelet homogenates suggests that this tissue cannot be used to develop a 'diagnostic test' for Parkinson's disease.

INTRODUCTION

The recent discovery of NADH CoQ_1 reductase (complex I) deficiency in Parkinson's disease (Schapira *et al.*, 1989, 1990*a*) has focused attention on the possible role of abnormal mitochondrial function in the pathogenesis of this disease. The first indication that abnormal mitochondrial function may be involved in Parkinson's disease came from analysis of the mechanism of action of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP produces parkinsonism in humans (Davis *et al.*, 1979; Langston *et al.*, 1983) and other primates (Burns *et al.*, 1983; Langston *et al.*, 1984) by inducing death of dopamine containing cells in the substantia nigra. MPTP is converted to its active metabolite 1-methyl-4-phenylpyridium (MPP^+) by glial monoamine oxidase B (MAO-B) (Salach *et al.*, 1984), an enzyme of the outer mitochondrial membrane. MPP^+ is accumulated within dopaminergic neurons by the dopamine re-uptake system (Javitch *et al.*, 1985) and is then concentrated into mitochondria by a specific-energy-dependent system capable of producing millimolar intramitochondrial concentrations of MPP^+ (Ramsay *et al.*, 1986). MPP^+ has been

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found to be a specific inhibitor of complex I both *in vivo* (Mizuno *et al.*, 1988) and *in vitro* (Nicklas *et al.*, 1985). The inhibition of complex I leads to a fall in adenosine triphosphate (ATP) production which ultimately results in cell death (Denton and Howard, 1987).

The demonstration of complex I deficiency in Parkinson's disease substantia nigra has provided a direct link between the idiopathic disease and the MPTP model. The recent demonstration that complex I deficiency is anatomically specific to the substantia nigra and disease specific for Parkinson's disease (Schapira *et al.*, 1990*c*) enhances the likelihood that this biochemical defect may be involved in the pathogenesis of Parkinson's disease. We have extended our initial studies on complex I deficiency in Parkinson's disease substantia nigra and have also examined mitochondrial function in skeletal muscle and platelet homogenates from parkinsonian patients.

METHODS

Patients

Muscle biopsies and platelet studies were performed with the full and informed consent of the patients and with the approval of the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, Queen Square, London. The diagnosis of Parkinson's disease was based on the presence of an akinetic rigid syndrome with asymmetric onset, a resting tremor and a positive response to apomorphine or L-dopa. Control muscle biopsies and blood for platelet studies were obtained from patients with no clinical evidence of neurodegenerative disease. Disease controls used in the platelet studies were also age-matched patients with no clinical evidence of any neurodegenerative disorder. This group included patients with multiple sclerosis, Guillian-Barré syndrome and carcinoma. Post-mortem brain samples were provided by the Parkinson's Disease Society Brain Bank. Parkinson's disease was confirmed pathologically in the contralateral fixed half brain by degeneration of the pars compacta of the substantia nigra and by the presence of Lewy bodies in nigral neurons. Control brain samples were removed from age-matched patients who had died without evidence of neurological or psychiatric disease and without pathology in the substantia nigra.

Brain samples

Samples of substantia nigra, tegmentum and cerebellum were removed from liquid nitrogen storage, weighed, homogenized in 9 vols of ice-cold medium (320 mM sucrose, 1 mM EDTA, 10 mM Tris, pH 7.4) using a glass-glass homogenizer. Protein concentration was measured as previously described (Lowry *et al.*, 1951). After three cycles of freeze-thawing, mitochondrial enzymes were assayed blind and in triplicate in a final volume of 1 ml at 25°C. NADH CoQ_1 reductase (complex I), succinate cytochrome *c* reductase (complexes II and III), cytochrome oxidase (complex IV) and citrate synthase were assayed by standard techniques (Schapira *et al.*, 1990*a*,*c*). Rotenone sensitive values were 40–65% of total NADH CoQ_1 reductase rates. For the succinate cytochrome *c* reductase assay the protein sample was maximally activated in the presence of succinate for 5 min at the assay temperature.

Muscle biopsies

Muscle biopsies were removed from left vastus lateralis under a brief, light general anaesthetic. Samples were processed for routine histochemistry and biochemical studies. Mitochondria were isolated within 90 min of biopsy as previously described (Holt *et al.*, 1989). Purified mitochondrial preparations were used immediately for polarographic determination of oxygen uptake and enzyme analysis. Assays of respiratory chain enzyme activities from purified mitochondria were performed as described for the brain samples. Rotenone sensitive rates for purified mitochondria were 64–82% of total NADH CoQ_1 reductase rates.

Platelet studies

Platelet homogenates were prepared from 30 ml of venous blood taken without tourniquet. Blood was mixed 9:1 with 3.8% (w/v) tri-sodium citrate, and platelet-rich plasma (PRP) separated at 12°C by centrifugation at 200 *g* for 20 min. The top three-quarters of the PRP was removed and centrifuged at 1000 *g* for 30 min. All subsequent steps were performed at 4°C. The resulting platelet pellet was gently resuspended in 15 ml Tyrodes buffer (pH 7.4) with 1 mM EDTA added, and the platelets pelleted by centrifugation at 1000 *g* for 15 min. The platelets were washed once more. The platelet pellet was gently resuspended on 0.75 ml ice-cold medium (250 mM sucrose, 1 mM EDTA, 10 mM Tris, pH 7.4) and disrupted with a glass-glass homogenizer. Mitochondrial respiratory chain enzyme activities were measured as for brain samples, but at 30°C. Rotenone sensitive rates were 23–37% of total NADH CoQ_1 reductase rates.

RESULTS

Brain samples

Table 1 summarizes the age, death to refrigeration and death to autopsy times of the various cohorts of control and parkinsonian patients. All patient groups were well matched to their controls for all these parameters. An additional 16 control and 10 parkinsonian substantia nigra, and 11 control and 12 parkinsonian cerebellum samples have been analysed and added to those studied previously (Schapira *et al.*, 1990*c*). The results of tegmentum analysis have not been reported previously. The only significant difference from control for any of the respiratory chain enzymes in any brain area examined was complex I activity in parkinsonian substantia nigra, which decreased by 37% of the control mean. Table 2 shows the correction of enzyme activities by citrate synthase activities to account for any variation in mitochondrial numbers between tissues. Complex I activity in parkinsonian substantia nigra remains the only enzyme activity significantly different from control. There was no correlation of complex I activity with the duration of the patients' Parkinson's disease (n = 14, r = 0.36), L-dopa treatment (n = 12, r = 0.12), death to freezing of brain (n = 17, r = 0.38). Controls also failed to show any correlation to complex I activity for storage parameters.

Muscle samples

Histochemical analysis of muscle biopsies from patients with Parkinson's disease showed no significant morphological abnormality. The substrates used in polarographic

TABLE 1 REGIONAL BRAIN ACTIVITIES OF MITOCHONDRIAL RESPIRATORY CHAIN ENZYMES

		Age (yrs)	*Death to refrigeration (min)*	*Death to autopsy (h)*	*NADH CoQ_1 rotenone sensitive*	*SCcR antimycin a sensitive*	*COX (k/min/mg)*	*CS*
Substantia nigra	C (n = 22)	72.4±13.4	151±76	16.6±7.2	3.90±1.24	11.86±2.54	1.09±0.38 (n = 19)	115.2±27.8
	PD (n = 17)	75.4±7.8	127±48	15.4±9.5	2.48±0.93*	11.01±2.08	1.02±0.31 (n = 13)	111.0±37.5
Tegmentum	C (n = 10)	75.9±8.2	187±71	16.3±8.5	2.59±0.86	8.65±1.73	0.76±0.17	92.1±16.3
	PD (n = 10)	75.8±8.2	133±50	14.7±8.1	2.55±0.48	8.46±1.85	0.89±0.20	108.0±26.4
Cerebellum	C (n = 16)	73.9±13.3	140±85	17.5±8.0	3.98±2.08	15.88±4.69	1.05±0.27	200.7±36.9
	PD (n = 16)	74.5±7.2	130±48	16.1±9.3	3.61±1.06	15.90±4.26	1.01±0.30	200.4±34.7

C = control; PD = Parkinson's disease; NADH CoQ_1 = NADH ubiquinone oxidoreductase (complex I); SCcR = succinate cytochrome *c* reductase (complexes II and III); COX = cytochrome oxidase (complex IV), CS = citrate synthase Results are expressed as mean ± SD. Enzyme activities are in nmol/min/mg total protein, unless stated, k is the first order rate constant. Significance relative to control by Student *t* test and Mann-Whitney U test. * $P = < 0.001$.

TABLE 2 REGIONAL BRAIN ACTIVITIES OF MITOCHONDRIAL RESPIRATORY CHAIN ENZYMES CORRECTED FOR CITRATE SYNTHASE ACTIVITIES

		NADH CoQ_1/CS (×100)	*SCcR/CS (×10)*	*COX/CS (×100)*
Substantia nigra	C (n = 22)	3.56±1.33	1.06±0.28	1.02±0.56 (n = 19)
	PD (n = 17)	2.50±1.08*	1.09±0.38	1.05±0.51 (n = 13)
Tegmentum	C (n = 10)	2.87±1.00	0.96±0.26	0.83±0.17
	PD (n = 10)	2.43±0.53	0.79±0.13	0.83±0.13
Cerebellum	C (n = 16)	1.99±0.97	0.82±0.31	0.55±0.19
	PD (n = 16)	1.81±0.45	0.80±0.21	0.51±0.16

*$P < 0.02$ Student *t* test and Mann-Whitney U test Abbreviations as for Table 1.

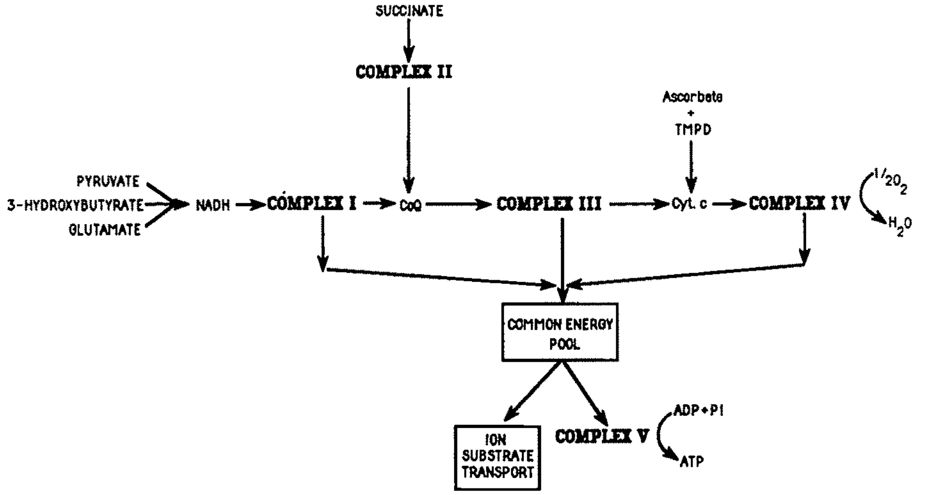

FIG 1. The mitochondrial respiratory chain. TMPD denotes *NNN'N'*-tetramethyl-*p*-phenylenediamine, CoQ coenzyme Q, Cyt. c cytochrome *c*, ADP adenosine diphosphate, and P_i inorganic phosphate. [Reprinted by permission of the *New England Journal of Medicine*, **323**, 37–42 (1990)].

analysis test oxygen utilization by each part of the respiratory chain (Fig. 1). Results of polarography of freshly isolated mitochondria from eight patients with Parkinson's disease (five untreated, three treated) did not show any significant difference in mitochondrial function when compared with age-matched controls (Table 3). In addition, spectrophotometric analyses of respiratory chain enzyme activities of muscle mitochondria from nine parkinsonian patients (five untreated, four treated) failed to show any difference when compared with age-matched controls (Table 4). There was no significant difference in mitochondrial function between treated and untreated patients by polarography or enzyme analysis.

Platelet studies

Two of the patients with Parkinson's disease were untreated. There was no difference in any of the mitochondrial respiratory chain activities between controls, disease controls

TABLE 3 POLAROGRAPHIC ANALYSIS OF SKELETAL MUSCLE MITOCHONDRIA

	Age	*Pyruvate*	*Glutamate*	*Succinate*	*Ascorbate/TMPD*
C (n = 5)	61.2±6.9	85±13	76±27	131±59	338±127
PD (n = 8)	62.4±7.6	83±12	85±29	119±19	227±113

TMPD = *NNN'N'*-tetramethyl-phenylenediamine. Results expressed as nmol O/min/mg mitochondrial protein

TABLE 4. MITOCHONDRIAL RESPIRATORY CHAIN ACTIVITIES OF SKELETAL MUSCLE MITOCHONDRIA

	Age (yrs)	*NADH CoQ_1 rotenone sensitive*	*SCcR antimycin a sensitive*	*COX (k/min/mg)*
C (n = 6)	63.2±1.9	95.6±37.9	204±72	25.2±11.4
PD (n = 9)	62.4±7.8	90.5±21.1	258±130	36.5±13.8

Results expressed as nmol/min/mg mitochondrial protein unless stated

TABLE 5 PLATELET MITOCHONDRIAL RESPIRATORY CHAIN ENZYME FUNCTION, ABSOLUTE AND CITRATE SYNTHASE CORRECTED

	Age (yrs)	*NADH CoQ_1 rotenone sensitive*	*SCcR antimycin a sensitive*	*COX (k/min/mg)*	*CS*	*NADH CoQ_1/CS (×100)*	*SCcR/CS (×10)*	*COX/CS (×100)*
Control n = 9	59.10±10.2	3.53±0.72	8.92±1.27	0.39±0.16	85.74±16.4	4.20±1.03	1.06±0.20	0.476±0.194
Disease control n = 6	57.17±10.4	3.65±1.11	9.67±3.43	0.52±0.09	86.82±3.81	4.21±1.32	1.11±0.38	0.595±0.095
PD n = 14	58.90±10.1	3.51±0.79	9.96±2.00	0.50±0.13	88.13±16.9	4.16±1.32	1.14±0.15	0.564±0.108

Results expressed as nmol/min/mg total protein unless stated. Abbreviations as for Table 1.

and Parkinson's disease patients (Table 5). Correction of activities for citrate synthase levels also failed to show any significant difference between the three groups.

DISCUSSION

These results support the anatomic specificity of complex I deficiency of the substantia nigra in Parkinson's disease. The results of this and a previous study (Schapira *et al.*, 1990*c*) have demonstrated normal mitochondrial function in parkinsonian cerebral cortex, cerebellum, caudate nucleus, putamen, medial and lateral globus pallidum, and tegmentum. Our finding of normal mitochondrial function in parkinsonian skeletal muscle is not surprising, therefore, bearing in mind the anatomic specificity of complex I deficiency in the CNS. Recent reports have indicated a decline of mitochondrial function in skeletal muscle with age (Trounce *et al.*, 1989) and so control and patient groups have been carefully matched in this respect in all our studies. Polarographic and enzyme analyses of respiratory chain activity in the skeletal muscle of our controls confirmed a decline with age, especially involving complex I (data not shown). Failure to use accurately age-matched controls could therefore produce misleading results.

Bindoff *et al.* (1989) studied muscle in five parkinsonian patients (one untreated) and four controls by enzyme analysis and found decreased levels of complexes I, II and IV in the parkinsonian group. However, our study using both polarographic and enzyme analysis does not support this finding. The deficiency of complexes I, II and IV observed in the previous study indicates a more comprehensive and non-specific decline in respiratory chain function that may be related to age and the relative immobility of parkinsonian patients. The controls used in our skeletal muscle study included orthopaedic patients matched for the relative immobility of our parkinsonian patients.

A recent study of skeletal muscle mitochondrial function in six patients with Parkinson's disease found normal respiratory chain function in one patient, pure complex I defects in two, complex I–III defects in a further two patients and low activity of complexes II–IV in one patient (Shoffner *et al.*, 1991). The level of complex I activity measured was 8%, 10%, 14% and 38% of control mean in affected patients. In patients with mitochondrial myopathy, muscle mitochondrial complex I activities below 20% of control, in our experience, have always been associated with severe clinical weakness, fatigue and a resting lactic acidosis. It is therefore surprising to see such low activities in parkinsonian patients, since in Parkinson's disease neither muscle weakness nor lactic acidosis are part of the normal clinical spectrum. The 16 controls used in their study varied in age from 6 yrs to 69 yrs, only four were 60 yrs or older, and the mean age of the group was 35 yrs. They found no decline in respiratory chain enzyme activity with age. This contrasts with the studies of Trounce *et al.* (1989) and Yen *et al.* (1989), as well as with the results of our own studies, in which a clear decline of complex I activity with age was shown.

The immediate assessment of respiratory chain function by both polarography and enzyme analysis in our study circumvented any effect that storage may have on enzyme function. In addition, the close age matching of our controls ensured against the production of an apparent age related complex I defect.

The results of our study on platelet homogenate mitochondrial function in 14 patients with Parkinson's disease are at variance with a previous study of 10 parkinsonian patients (Parker *et al.*, 1989). We used platelet homogenates to determine respiratory chain enzyme activities, whereas Parker *et al.* (1989) used mitochondrially enriched preparations obtained by platelet-phoresis and disruption using a nitrogen bomb. Nevertheless, the degree of complex I deficiency suggested in parkinsonian platelets by the latter group (about 50%) should be detectable by our techniques. Although the complex I assay was on average only 31% rotenone-sensitive, triplicate assays of each sample fell within 10% of each other, reflecting the reproducibility of the analysis. Our adjustment of variations in citrate synthase activity allows for correction of any differences in mitochondrial content between samples. The use of platelet homogenate activities corrected for citrate synthase activities circumvents the potential problems associated with enriched mitochondrial preparations which may have variable degrees of contamination by other subcellular compartments. Nevertheless it is still conceivable that a mild to moderate defect of platelet complex I activity in patients with Parkinson's disease may not be detected in homogenates.

Our findings of normal skeletal muscle and platelet mitochondrial function in Parkinson's disease corresponds with the specificity of the complex I defect to the substantia nigra within the brain, and with the concept of Parkinson's disease as a neurodegenerative disorder. Our failure to demonstrate complex I deficiency in platelet homogenates indicates that this tissue could not be easily used, if at all, as a 'diagnostic test' for Parkinson's disease.

The relationship of the substantia nigra complex I deficiency to the cause of Parkinson's disease is as yet unclear. This defect may be a primary phenomenon, or the end result of endogenous or exogenous toxic activity. In either case, the complex I deficiency will result in decreased ATP synthesis leading to cell death.

Current hypotheses for the etiology of Parkinson's disease must take account of data suggesting contributions from both genetic and environmental factors. The recent description of autosomal dominantly inherited Parkinson's disease in a large American-Italian family (Golbe *et al.*, 1990) implies that this disorder can potentially be induced by a nuclear gene defect. The MPTP model clearly demonstrates the ability of an environmental agent to cause Parkinson's disease. The recent identification of abnormal xenobiotic metabolism in patients with Parkinson's disease (Steventon *et al.*, 1989) suggests that this disorder may develop as a result of an inherited defect involving abnormal metabolism of environmental chemicals.

Complex I deficiency may play a pivotal role in the chain of events culminating in nigral cell death. Complex I comprises 26 polypeptides, seven of which are encoded by mitochondrial DNA (mtDNA). Mitochondrial DNA is maternally inherited and so a mitochondrially encoded defect could not be directly involved in a disorder inherited in an autosomal pattern. In addition, Parkinson's disease itself does not exhibit maternal inheritance. In agreement with this, two studies have examined mtDNA in parkinsonian brain by restriction fragment length polymorphism (RFLP) analysis and failed to show any significant deletions in any region including substantia nigra (Lestienne *et al.*, 1990; Schapira *et al.*, 1990*b*). Deletions of mtDNA have been seen after amplification by the polymerase chain reactions in parkinsonian striatum and cortex, but similar deletions were present in age-matched controls (Ikebe *et al.*, 1990) and so are likely to be related simply to ageing. Although mtDNA may not play any part in the inheritance of Parkinson's disease, mutations of mtDNA may be induced by certain toxic processes. Mitochondrial DNA lacks the protection of histones and repair enzymes and so is susceptible to damage from free radicals (Richter *et al.*, 1988) which could lead to defects of mtDNA encoded products. The fact that more than half the polypeptides encoded by mtDNA are part of complex I, renders this protein of the respiratory chain potentially the most vulnerable to such an effect. In addition, the mitochondrially encoded products of complex I are important functional elements of complex I, in particular the NDI gene product which is involved in the binding site of the mitochondrial inhibitors rotenone (Earley *et al.*, 1987), a pethidine analogue (Werner, 1989) and possibly MPP$^+$ (Ramsay *et al.*, 1991). There is evidence of an active toxic process involving oxidative damage in the substantia nigra of patients with Parkinson's disease (Dexter *et al.*, 1989) and this could induce mtDNA abnormalities not detected by RFLP analysis. The mitochondrial respiratory chain is an important source of superoxide radicals and this production is stimulated by inhibitors such as rotenone (Takeshige and Minakami, 1979). Thus complex I inhibition itself may lead to the oxidative damage seen in parkinsonian substantia nigra. However, there is some evidence that free radicals themselves can impair complex I activity (Hillered and Ernster, 1983). This close association between complex I inhibition and free radical production has been confirmed in the MPTP/MPP$^+$ model (Cleeter *et al.*, 1992). It has been shown that the irreversible inhibition of complex I function induced by MPP^+ can generate free radicals which in turn irreversibly and specifically inhibit complex I. This suggests that a primary defect in complex I could produce a cycle of free radical production and complex I inhibition, and account for the findings of complex I inhibition and oxidative stress in parkinsonian substantia nigra.

The finding of complex I deficiency in Parkinson's disease and its anatomic selectivity for the substantia nigra provides a direct link between the idiopathic disease and the toxin (MPTP) model. The recent description of normal complex I activity in multiple system atrophy (MSA, a disorder involving severe degeneration of nigral neurons) indicates that this biochemical defect is not simply a secondary phenomenon of cell death (Schapira *et al.*, 1990*c*). Furthermore, the fact that the MSA patients studied were taking L-dopa in amounts equivalent to those of parkinsonian patients shows that complex I deficiency is not a side-effect of treatment. All these factors enhance the possibility that complex I deficiency plays an important role in the pathogenesis of Parkinson's disease. Further work needs to be focused on the molecular basis of this defect and the possible mechanisms that lead to it. The relationship between the complex I deficiency and oxidative stress also needs to be explored further.

The results of this study suggest that neither muscle nor platelet homogenates from patients with Parkinson's disease are suitable for the investigation of complex I deficiency in this disorder.

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A MOLECULAR GENETIC STUDY OF FOCAL HISTOCHEMICAL DEFECTS IN MITOCHONDRIAL ENCEPHALOMYOPATHIES

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SUMMARY

Using *in situ* hybridization and histochemistry we have studied muscle biopsy samples from eight patients with mitochondrial encephalomyopathies and known defects of mitochondrial DNA (mtDNA). In four patients with heteroplasmic mtDNA deletions there were focal accumulations of deleted mtDNA and its transcripts within ragged red fibres (RRF). In one of these, a probe designed specifically to detect deleted mtDNA identified abundant deleted mtDNA and its fusion transcript in RRF and lesser accumulations in non-ragged red cytochrome oxidase (COX) deficient fibres. A further patient with a deletion involving the heavy strand promoter region showed accumulation of deleted mtDNA and light strand transcripts in RRF, but concomitant depletion of all heavy strand transcripts. In all patients with deletions, normal mtDNA transcripts were depleted in COX deficient fibres irrespective of ragged red change. Deleted mtDNA was rare or absent in normal fibres. Within RRF, COX activity was more profoundly impaired in patients with deletions involving COX subunits.

In two patients heteroplasmic for the base pair (bp) 3243 mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and strokelike episodes (MELAS), RRF contained a great excess of mtDNA and transcripts of all species. Some RRF showed excess COX activity. Non-ragged red COX deficient fibres showed equal increases of ribosomal RNA (rRNA) and messenger RNA, suggesting that focal biochemical defects were not associated with a quantitative defect of transcription termination at the 3′ end of the 16S rRNA which might be predicted. A patient heteroplasmic for the bp 8344 mutation (associated with myoclonic epilepsy and ragged red fibres: MERRF) showed subnormal COX activity within RRF, although the tissue distribution of mtDNA and its transcripts was similar to that seen with the bp 3243 mutation. Within mitochondrial encephalomyopathies, the relationships between the distribution and expression of abnormal mtDNA and the focal biochemical consequences are complex and heterogeneous.

INTRODUCTION

Several defects of mitochondrial DNA (mtDNA) have been described in association with human neurological disorders. Deletions of a proportion of muscle mtDNA are observed in about 40% of adult patients with mitochondrial myopathies (Holt *et al.*, 1988, 1989*a*; Zeviani *et al.*, 1988; Moraes *et al.*, 1989; Goto *et al.*, 1990*a*). Most deletions are situated in the larger arc of mtDNA between the origins of replication of the light strand (L strand) and heavy strand (H strand) (O_H, O_L respectively); only four patients have been described with deletions of the smaller arch between O_H and O_L (Holt *et al.*, 1989*a*; Johns and Cornblath, 1991; Moraes *et al.*, 1991). The presence of a single mitochondrial DNA deletion is invariably accompanied by progressive

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external ophthalmoplegia (PEO) in patients with neurological disease. Some of these have one or more other elements of the Kearns Sayre syndrome [KSS: PEO, onset before 20, pigmentary retinopathy and variable ataxia, heart block or elevated CSF protein concentration (Berenberg *et al.*, 1977)].

Point mutations of mtDNA are associated with Leber's hereditary optic neuropathy and some of the mitochondrial encephalomyopathies. Of the encephalopathies, a base pair (bp) 8344 A → G point mutation in the lysine transfer RNA ($tRNA^{Lys}$) gene is associated with the syndrome of myoclonic epilepsy and ragged red fibres (MERRF: Shoffner *et al.*, 1990) and appears to be present in the majority of patients with this syndrome (Berkovic *et al.*, 1991; Hammans *et al.*, 1991; Zeviani *et al.*, 1991*a*). A bp 3243 A → G mutation in one of the leucine tRNA genes ($tRNA^{Leu(UUR)}$) is observed not only in the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS; Goto *et al.*, 1990*b*; Kobayashi *et al.*, 1990) but also in other mitochondrial myopathies with or without encephalopathy (Hammans *et al.*, 1991). A further point mutation of the $tRNA^{Leu(UUR)}$ gene has been described in maternally inherited myopathy and cardiomyopathy (Zeviani *et al.*, 1991*b*). In these disorders the mutant mtDNAs coexist with normal mtDNA in tissues (heteroplasmy).

The pathogenesis of mitochondrial myopathies is heterogeneous and not fully understood. Respiratory chain function can be studied histochemically using stains for cytochrome oxidase (COX) and succinic dehydrogenase (SDH) activity. Succinic dehydrogenase is exclusively encoded by nuclear DNA, while the three largest subunits of COX are mtDNA encoded. Biochemical investigations of pooled mitochondria, showing defects of the respiratory chain and oxidative phosphorylation system in many patients with these disorders, often do not reflect focal defects apparent on histochemistry. Similarly, studies of mtDNA and mtRNA which assay extracted nucleic acids from muscle or other tissues do not reflect the focal distributions of populations of normal, deleted and mutant DNA (NmtDNA, DmtDNA and MmtDNA, respectively).

The distributions of mtDNA and transcripts within tissues may be examined by means of *in situ* hybridization. Using this technique Mita *et al.* (1989) studied a single patient with a deletion of mtDNA. Focal proliferation of DmtDNA was demonstrated within ragged red fibres (RRF), whereas NmtDNA was depleted in these fibres, which were universally COX negative. These findings differed from those of Shoubridge *et al.* (1990) who studied two patients with deletions and found that NmtDNA transcripts were distributed uniformly throughout muscle. They proposed that DmtDNA was functionally dominant, causing a focal biochemical defect even in the presence of normal amounts of NmtDNA. Moraes *et al.* (1991) reported two patients with a deletion involving part of the heavy strand promoter (HSP) region. *In situ* hybridization studies of mtRNA showed increased levels of L-strand transcripts in RRF. Heavy-strand transcripts, either inside or outside the deleted region, were described as not overexpressed. The distribution of mtDNA was not studied. The presence of COX negative fibres without mitochondrial proliferation was noted.

Because the bp 8344 mutation lies within the $tRNA^{Lys}$ gene, dysfunction of $tRNA^{Lys}$ has been proposed to be of pathophysiological significance in MERRF, consistent with observations of multiple respiratory chain defects in this syndrome (Wallace *et al.*, 1988; Shoffner *et al.*, 1990). Although the bp 3243 mutation may influence $tRNA^{Leu(UUR)}$ function, it is also embedded in the middle of a tridecamer sequence necessary for the formation of 3′ ends of 16S ribosomal RNA (rRNA) *in vitro*. This termination site is the major determinant controlling the relative synthesis of rRNA compared with messenger RNA (mRNA) molecules transcribed from the H-strand (Christianson and Clayton, 1986, 1988). Hess *et al.* (1991) demonstrated severe impairment of transcription termination in the presence of the bp 3243 mutation *in vitro*, correlated with reduced affinity of the partially purified termination protein (designated mtTERM) for the mutant template. It was suggested that this mutation may cause inability to produce the correct type or quantity of rRNA relative to other mitochondrial gene products.

We have examined the distribution of histochemical defects, mtDNA, and mtDNA transcripts in muscle in eight patients with mitochondrial encephalomyopathies and different defects of mtDNA.

PATIENTS

The clinical, genetic and biochemical data are summarized in Table 1. Sites of genetic defects are shown in Fig. 1. Patient numbers used previously (Holt *et al.*, 1989*a*) are as shown in Table 1, for ease of reference they are referred to here as cases A–H. Five had PEO with one or more other manifestations of the Kearns-Sayre syndrome and deletions of mtDNA (patients A–E), one (A) with the 'common' deletion (Holt *et al.*, 1989*a*; Schon *et al.*, 1989). Two patients had the bp 3243 mutation; one (patient F) had myopathy alone and one (patient G) a typical MELAS phenotype. One patient (H) had the syndrome of MERRF with the bp 8344 mutation.

TABLE 1. CLINICAL, HISTOCHEMICAL, BIOCHEMICAL AND GENETIC FEATURES OF PATIENTS STUDIED

Patient no.	*Clinical features*	*%RRF/%COX negative (% both RRF and COX negative)*	*Genetic defect*	*Biochemical defect involving respiratory chain complexes*
Deletions (repeat*)				
A (17)	ORMDfAH	25/39 (23)	8470-13459 (13)	I–IV
B (95)	ORMDfAH	3/4 (1)	7439-13476 (1)	N
C (85)	OM	17/12 (8)	12103-14421 (10)	I
D (16)	ORMDfA	6/0 (0)	12103-14421 (10)	I
E (2)	ORMDf	11/11 (4)	504-5451 (8/9)	I
Point mutations				
F (78)	RSStA	3/0 (0)	bp 3243 (A→G)	N**
G (47)	M	27/5 (1)	bp 3243 (A→G)	I
H (99)	ADfMyS	1/3 (0.3)	bp 8344 (A→G)	n d.

* Number of nucleotides in direct repeat flanking deleted region. **Mother was also heteroplasmic for bp 3243 mutation, and had a complex I defect on polarography. O = ophthalmoplegia; R = retinopathy; M = limb weakness; Df = deafness; A = ataxia; H = heart block; My = myoclonus; S = seizures; St = stroke-like episodes; N = normal; n.d. = not done. Biochemical defects refer to polarographic data (Holt *et al.*, 1989*a*). Patient numbers are the same as those in Petty *et al.* (1986); Holt *et al.* (1989*a*); Hammans *et al.* (1992).

METHODS

Mitochondrial DNA from muscle was examined for deletions or point mutations as previously described (Holt *et al.*, 1988, 1989*a*; Hammans *et al.*, 1991, 1992). In patient E the deletion breakpoint was mapped and subsequently amplified and sequenced directly (McShane *et al.*, 1991) using primers bp 5901–5920 (5′CAACGGTCGGCGAACATCAG3′), bp 520–268 (5′TGTCTGCACAGCCACTTTC3′) and bp 371–390 (5′CTAACACCAGCCTAACCAGA3′). Oligonucleotides (Table 2, Fig. 1) were labelled

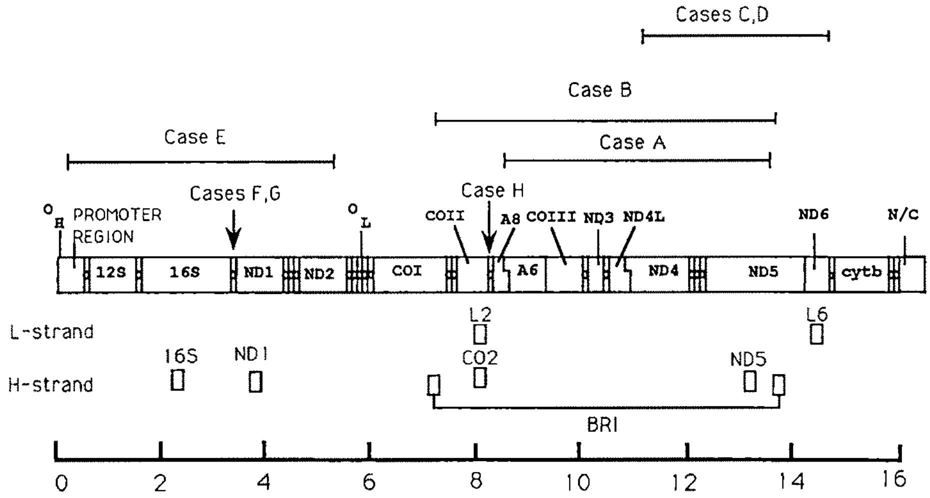

FIG. 1. Linearized mitochondrial genome showing sites of deletions in patients A–E, and sites of A to G point mutations in patients F, G, H. Sites of oligonucleotides probes are shown. H-strand probes are complementary to mRNA transcribed from the H-strand; L-strand probes are complementary to mRNA transcribed from the L-strand.

TABLE 2 OLIGONUCLEOTIDE PROBES USED FOR *IN SITU* HYDRIDIZATION

ND1	Bp 3377–3330 48 bases	5′-TCGGTAAGCATTAGGAATGCCATTGCGATTAGAATGGGTACAATGAGG-3′	Heavy strand
CO2	Bp 8014–7967 48 bases	5′-TACTACTCGATTGTCAACGTCAAGGAGTCGCAGGTCGCCTGGTTCTAG-3′	Heavy strand
ND5	Bp 13216–13163 48 bases	5′-CAGACTGCTGCGAACAGAGTGGTGATAGCGCCTAAGCATAGTGTTAGA-3′	Heavy strand
16S	Bp 2576–2541 36 bases	5′-TACCGCGGCCGTTAAACATGTGTCACTGGGCAGGCG-3′	Heavy strand
BRI	Bp 13488–(13476 = 7439)–7427 25 bases	5′-AGGTATTCCTGCTTTTATGTATACG-3′	Heavy strand
L2	Bp 7890–8014 35 bases	5′-ACCTGCGACTCCTTGACGTTGACAATCGAGTAGTA-3′	Light strand
L6	Bp 14351–14385 35 bases	5′-TCTTTCACCCACAGGACGAATCCTACCTCCATCGC-3′	Light strand
RUB	Nonsense 48 bases	5′-GTAGGCATAATGAGCGTAGTCCTCATAGTTAGTGGACTCGAGTGGTAT-3′	

with [^{35}S]dATP using an Amersham 3′ end labelling kit. Probes were purified using Nensorb-20 cartridges; 5–10×10^5 d.p.m. of probe were used per slide. Muscle was frozen immediately after biopsy; serial 8 μm sections were thaw mounted onto gelatin and chrome alum-coated slides, each mounted adjacent to a section of control muscle. For *in situ* hybridization, the muscle was fixed in fresh 4% paraformaldehyde in phosphate buffered saline (PBS) for 5 min and then washed. For DNA studies the sections were treated with ribonuclease A (RNase) in 2×SSC (0.3 M NaCl/0.03 M sodium citrate, pH 7.0). Prehybridization was for 1 h in 50% formamide, 4×SSC, 500 μg/ml single stranded DNA, 250 μg/ml yeast tRNA, 1×Denhardt's solution and 10% dextran sulphate. For detection of DNA the sections were denatured in 70% formamide, 2×SSC at 75°C for 5 min. Sections were incubated overnight with labelled probes in 45 μl prehybridization buffer at 37°C under parafilm coverslips in a humid chamber. Additional sections were treated with RNase or with a nonsense probe (RUB; Table 2) to act as controls.

The slides were washed four times in 1×SSC for 15 min at 55 °C, twice for 30 min at room temperature, dipped briefly in distilled water and 70% ethanol, and air dried before being coated with Ilford K5 emulsion diluted 50% with water. The slides were developed between 2 d and 28 d later to detect RNA or 7 d and 40 d to detect DNA, and then stained by the modified Gomori trichrome method.

Sections adjacent to those undergoing *in situ* hybridization were stained histochemically; counting of RRF and COX negative fibres was performed on serial sections as described previously (Holt *et al.*, 1989*a*). Sections used for comparing histochemical data with *in situ* hybridization data were within five sections (40 μm). Some slides on which *in situ* hybridization had been performed were analysed for fibre grain density using PC based image analysis software (Synoptics Ltd, UK). A microscopic field was selected for analysis and the corresponding field was identical on serial SDH, COX, ATPase stained sections exposed to pertinent oligonucleotide probes. Each microscopic field was digitized to 512×512 pixels and corrected for shading effects introduced by the microscope and charge coupled device (CCD) camera. Fibre boundaries were outlined using a mouse. Grains were identified using a threshold derived from the mode intensity to allow for variation in fibre staining. The grain to fibre area ratio was calculated for each fibre. For analysis of NmtDNA transcripts within RRF, grain density was calculated as a ratio between the RRF and adjacent type 1 and type 2 fibres of normal histochemical appearance, in order to avoid errors originating from variation of sensitivity of the technique within and between sections. Differences in grain density between paired RRF and adjacent normal fibres of same fibre type were tested using the sign test.

RESULTS

Quantitative histochemical data are shown in Table 1. Minimal grain density was observed over sections exposed to RNase without being denatured prior to hybridization with probes, or in sections hybridized to the nonsense probe. Moderate non-specific signal was observed when sections were treated to detect mtDNA, sufficient in density to obscure partly the different grain densities between type 1 and type 2 fibres, but minimal background was observed in detecting mtRNA and fibre types were clearly distinguished. In early experiments some sections were stained for COX activity prior to *in situ* hybridization. These sections showed silver grain density proportional to COX activity with control probes or unlabelled probe suggesting chemographic interference with the photographic emulsion (Haase, 1987). Sections with prior staining for COX activity were thus excluded from the analysis. For each slide used for *in situ* hybridization, histochemically normal fibres from diseases muscle were compared with fibres of similar type from control muscle, and were found to have a similar signal.

TABLE 3. GRAIN DENSITY OVER FIBRES USING A PROBE TO DETECT NmtDNA TRANSCRIPTS, EXPRESSED AS A PERCENTAGE OF THE GRAIN DENSITY OVER AN ADJACENT NORMAL TYPE 1 FIBRE

	Median value			
Fibre type	*Patient A*	*Patient B*	*Patient C*	*All three patients*
RRF (type 1)	19.3*	18.7*	26.1*	19.3*
(No. of fibres: range)	(14; 4.3–47.6)	(11; 7.1–27.5)	(8; 4.7–43.2)	(33; 4.3–47.6)
RRF (type 2)	31.9*	22.7	16.7*	17.9*
(No. of fibres: range)	(6; 11.6–60.0)	(3; 11.7–23.0)	(8; 3.2–25.2)	(17; 3.2–60.0)
Normal type 2	61.5	44.5	53.3	54.0
(No. of fibres: range)	(11; 26.0–79.5)	(10; 26.0–76.5)	(13; 21.4–21.0)	(34; 21.4–79.5)

* Difference in grain density between RRF and paired normal adjacent fibres of same fibre type reaches significance ($P < 0.05$, sign test).

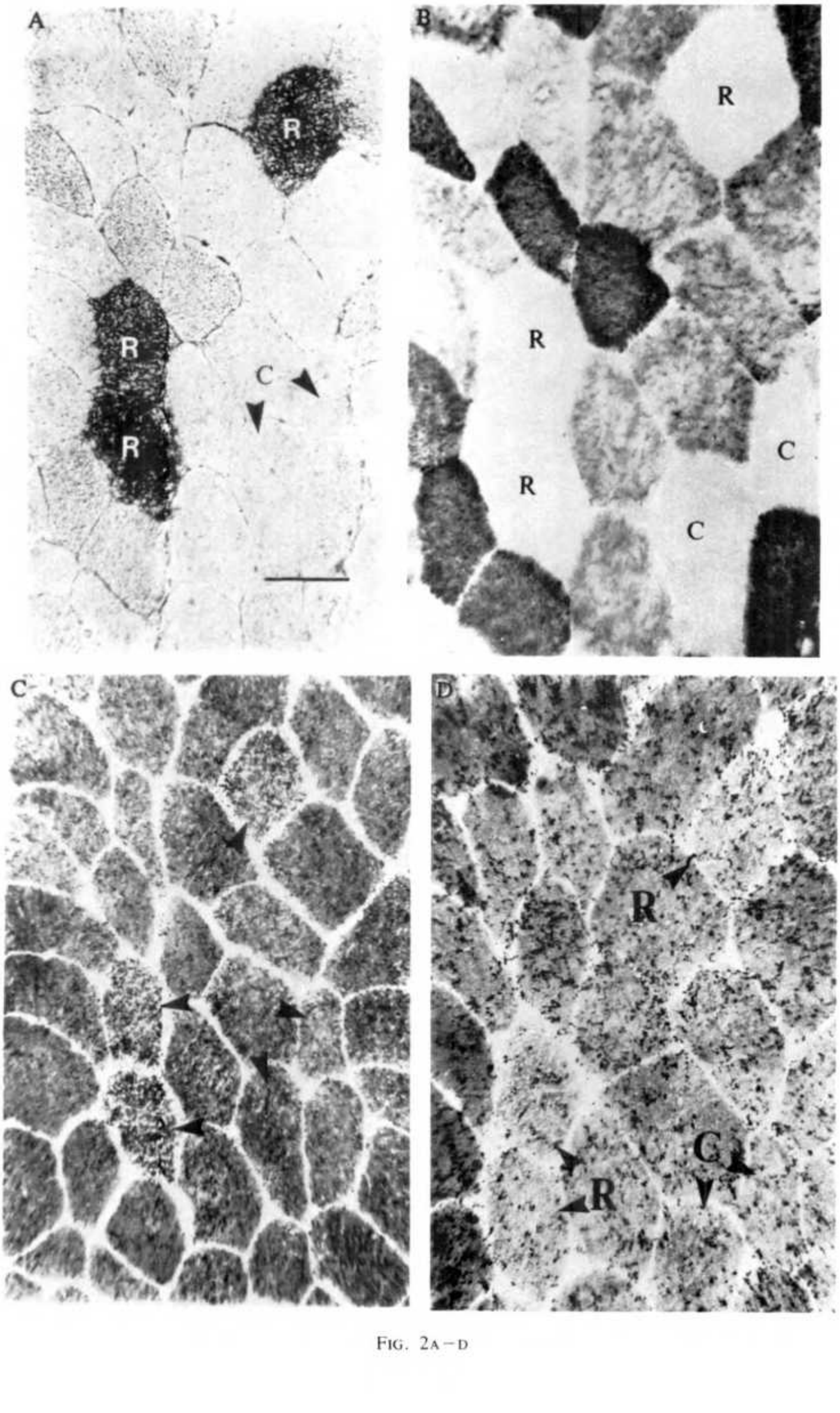

FIG. 2A–D

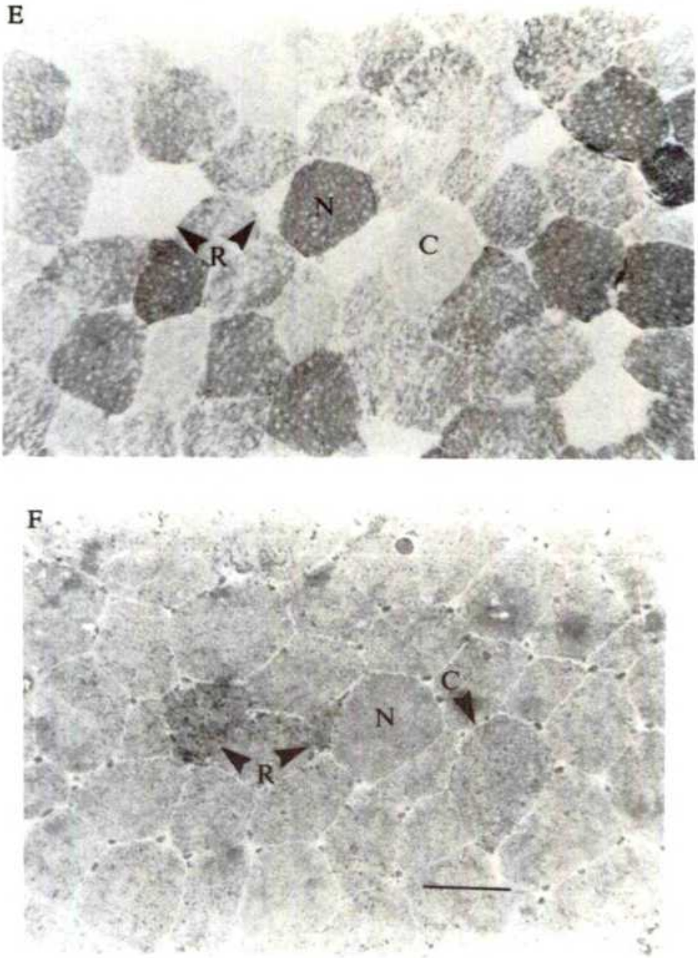

FIG. 2. Serial muscle sections from patient B. Bar represents 100 μm. Variation in fibre staining in sections exposed to *in situ* hybridization in some figures is due to variable uptake of the modified Gomori trichrome stain. A, SDH stain showing excess activity in three type 1 RRF (R) and normal activity in two other fibres (C). B, COX stain showing absent activity in the three RRF, and COX deficiency in the two fibres marked C. C, *in situ* hybridization with probe ND1, detecting ND1 mRNA from both DmtDNA and NmtDNA. Excess transcripts are observed within RRF. Arrows point to RRF and COX negative fibres. D, *in situ* hybridization with probe ND5, detecting ND5 transcripts of NmtDNA only. Both RRF and non-RRF COX deficient fibres show lower signal than adjacent normal fibres. Arrows point to fibres. E, COX staining showing RRF (R), a non-ragged red COX deficient fibre (C) and a histochemically normal type 1 fibre (N). F, *in situ* hybridization with probe BRI, detecting fusion transcripts from DmtDNA. High grain density is observed over RRF with variable but lesser amounts over non-RRF COX deficient fibres. Low or absent signal is present over normal fibres.

Patients with deletions of mtDNA

Patient A. Examination of SDH and COX stained sections showed RRF and COX negative fibres. All RRF showed absent or profoundly deficient COX activity; some COX negative fibres were not ragged red but showed mild or moderate increases in SDH staining. The distribution of mtDNA was examined using probes 16S, ND5 and

CO2. Probes 16S and CO2 detected a marked excess of total mtDNA in RRF, in a predominantly subsarcolemmal distribution. ND5, which detected only NmtDNA, showed a near uniform distribution throughout the section, although type 1 fibres contained a higher grain density than type 2 fibres.

The distribution of mtRNA was examined using the same probes and probe L6. An extremely high steady state level of 16S rRNA was suggested by high signal with 16S probe after only 2–3 d exposure. Ragged red fibres showed high signal with probes 16S, CO2 or L6 in a predominantly subsarcolemmal distribution though some RRF had high signal uniformly across the fibre. Seventeen pairs of RRF with adjacent normal fibres were identified from a section exposed to probe CO2 to detect COII mRNA and examined using image analysis. Grain density over RRF exceeded that over normal fibres by a median ratio of 6.5 (range 3.3–36; RRF show a significant increase over paired adjacent normal fibres, $P < 0.01$). ND5 mRNA, transcribed exclusively from NmtDNA, showed subnormal levels within RRF; these were quantified using image analysis (Table 3). In common with RRF, non-ragged red COX negative fibres showed subnormal levels of NmtDNA transcripts in comparison with adjacent normal fibres.

Patient B. Similar findings from *in situ* hybridization and histochemical studies were noted in patient B as in patient A, though the prevalence of RRF and COX negative fibres was somewhat lower (Table 1). Ragged red fibres contained significantly reduced levels of NmtDNA transcripts, which were quantified (Table 3). The absence of a direct repeat flanking the deletion allowed the design of a probe (BRI) to detect only DmtDNA or fusion transcripts derived from DmtDNA, since the probe contained only 13 bases complementary to the normal type sequence (at each end of the deletion). Sections exposed to probe BRI showed specific signal with little background. Ragged red fibres showed dramatic accumulations of DmtDNA and its fusion transcript. Normal fibres showed rare or absent DmtDNA transcripts but COX negative or COX deficient fibres showed the presence of DmtDNA transcripts even in the absence of ragged red change. As in patient A, these non-ragged red COX negative fibres showed subnormal levels of NmtDNA transcripts in comparison with adjacent normal fibres (*see* Fig. 2 and Table 3).

Patients C and D. These patients, with smaller deletions, showed different histochemical findings to patients A and B. Ragged red fibres were in excess of COX negative fibres overall. Patient C had more RRF than patient D; some were COX negative and these were almost exclusively type 2 fibres. In adjacent sections most other RRF showed subnormal but not absent COX activity with occasional hyperdense staining (Fig. 3). In patient D some RRF showed profoundly deficient COX activity, but it was never undetectable.

Studies using *in situ* hybridization were, however, similar to patients A and B. There was excess total mtDNA and mtRNA in RRF (probes 16S, CO2, L6; Fig. 3), whereas NmtDNA was distributed almost uniformly. Ragged red fibres and COX deficient fibres showed a decrease in prevalence of NmtDNA transcripts detected with probe ND5 (Table 3).

Patient E. Amplification across the deletion breakpoint and subsequent sequencing identified a deletion of 4939 bp flanked by a 9 bp imperfect repeat existing at bp 504–512 and bp 5443–5451 (Figs 1, 4, 5). Muscle histology showed considerable variation in muscle fibre diameters ranging from 20 μm to over 200 μm. Internal nuclei were common and there were occasional necrotic fibres. Most RRF showed absent or markedly reduced

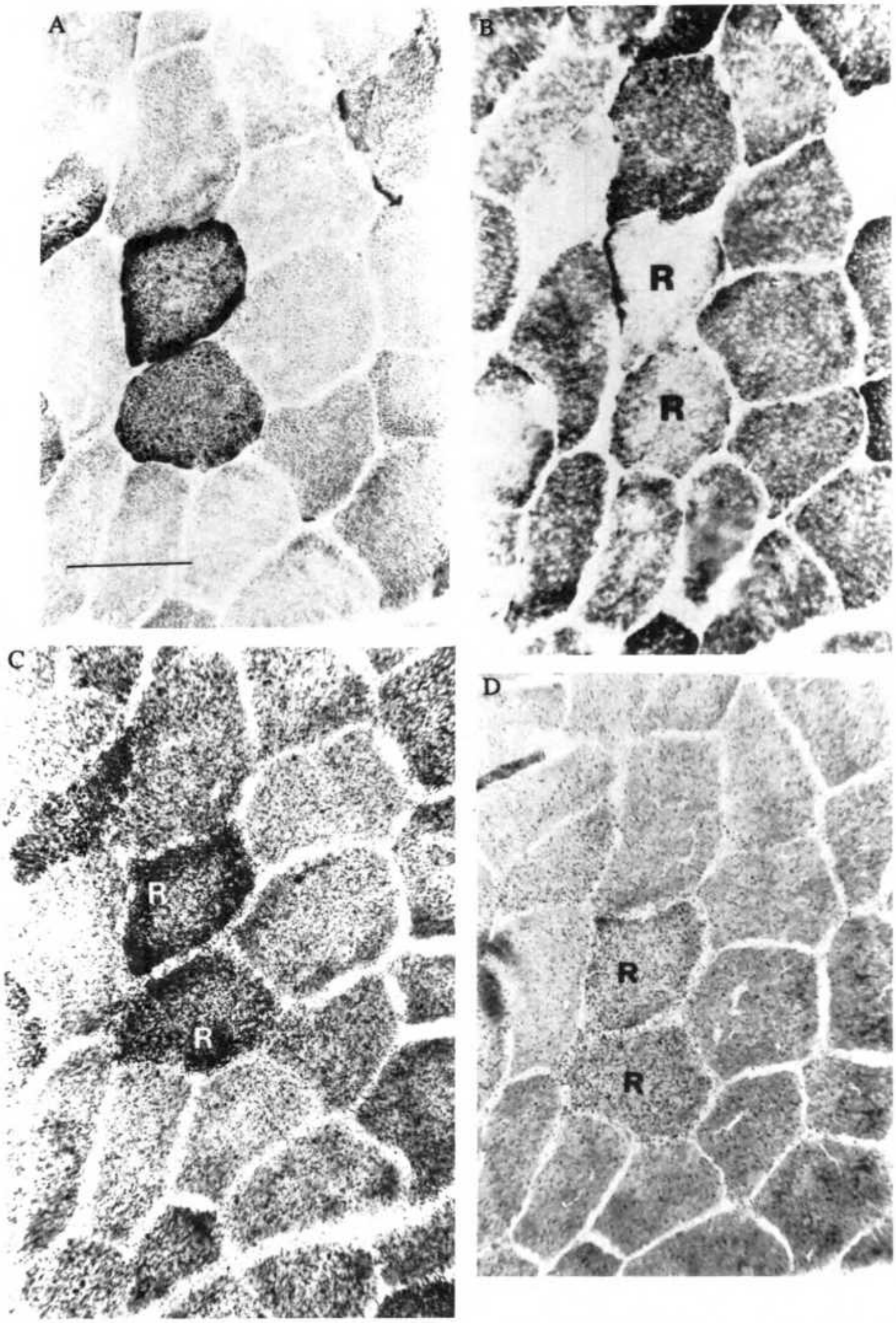

FIG. 3. Serial muscle sections from patient C. Bar represents 100 μm. A, SDH stain demonstrating increased activity in two RRF, and part of two others. B, COX stain demonstrating reduced but not absent COX activity within the two central RRF (R). COX activity is particularly preserved in the subsarcolemmal regions. The other RRF show absent or reduced COX activity. C, *in situ* hybridization with probe 16S, detecting 16S rRNA transcribed from both DmtDNA and NmtDNA. Excess signal is observed within RRF. D, *in situ* hybridization with probe CO2, detecting COII mRNA transcribed from both species of mtDNA; excess is detected within RRF.

		Deleted reading frames	Deleted tRNAs
	5′ 3′		
	▼504 ▼512	mtTF1 binding site	Valine
Normal non-coding sequence	CCGCCCA **TCCTACCCA** GCACAC	H Strand promoters	Leucine 1(UUA/G)
Patient sequence	CCGCCCA TCCTACCCA CACTCA	12S, 16S rRNAs	Isoleucine
Normal ND2 sequence	AC CCCAT**TCCTCCCCA** CACTCA	ND1, ND2	Glutamine
	▲5443 ▲5451	mtTERM binding site	Methionine
			Phenylalanine

FIG. 4. Sequence of the deletion breakpoint in patients E. The 8/9 imperfect repeat is shown in bold. mtTF1 = mitochondrial transcription factor 1. ND = complex 1. The function of the mtTERM binding site is described in the text.

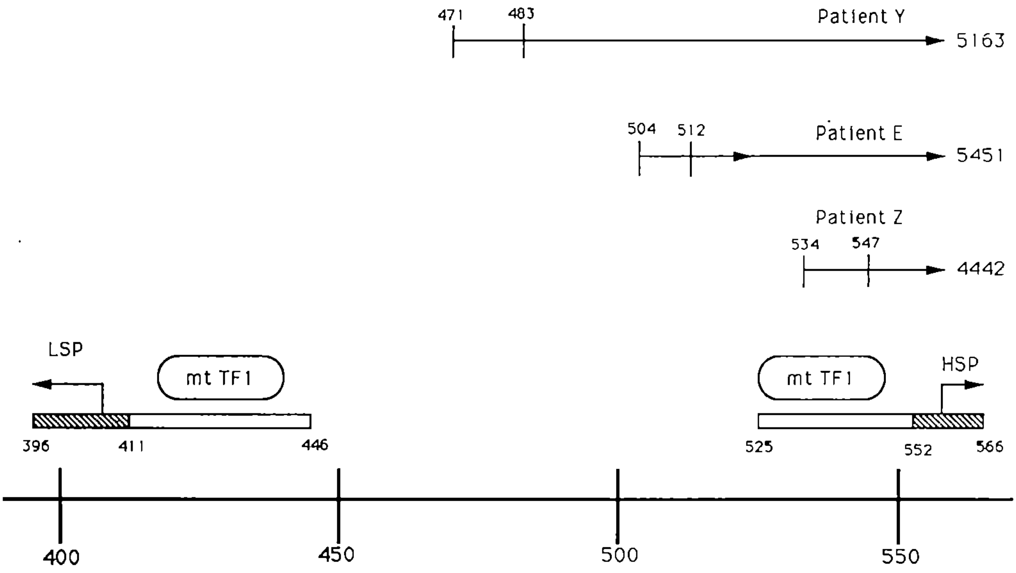

FIG. 5. Schematic diagram showing the mitochondrial promoter region. The L-strand promoter and H-strand promoter (LSP and HSP, respectively) are shown according to positions defined by Topper and Clayton (1989). The boxes denote regions required for *in vitro* transcription of human mtDNA. The shaded boxes denote the start site regions and the unshaded boxes upstream regulatory elements which include the mtTF1 binding sites. Deletion Z was described by Moraes *et al.* (1991), deletion E refers to our patient E and deletion Y to the patient of Johns and Cornblath (1991). The positions of direct repeats flanking the deletions are shown between vertical bars.

COX activity. There were occasional COX negative fibres with mild to severe depletion of mitochondria as judged by succinic dehydrogenase histochemistry when compared with normal fibres. These fibres were all type 2 fibres.

Total mtDNA (CO2 and ND5 probes) showed markedly increased signal within RRF in contrast to adjacent normal fibres (Fig. 6). Most of the excess signal was located in subsarcolemmal regions though it was also increased internally. NmtDNA (16S probe) showed near uniform distribution.

Heavy-strand transcripts were detected using 16S, ND5 and CO2 probes. In RRF the signal from both ND5 and CO2 mRNA and from 16S rRNA was markedly reduced in comparison with adjacent normal fibres (Fig. 6). Light-strand transcripts were detected using probes L2 and L6. Both probes showed increased signal in RRF with a subsarcolemmal distribution reflecting that of total mtDNA.

The fibres which were COX negative fibres with depletion of mitochondria showed low abundance of total mtDNA, and generally were smaller than normal fibres (Fig. 6). The majority of the mtDNA within these fibres appeared to be of the deleted species, while H-strand transcripts, whether detected by the CO2, ND5 or 16S probes, were absent or rare. Light-strand transcripts were of near normal abundance.

Patients with point mutations of mtDNA

Patients F and G. In patient F, there were infrequent RRF on histochemical analysis. These usually showed patchily dense COX activity, though some were COX deficient. Some other fibres showed profound COX deficiency, but none was COX negative; these fibres showed mild or moderate increases in SDH staining without ragged red change. Total mtDNA (probe 16S, CO2, ND5) showed marked accumulation within RRF. In other fibres with increased SDH staining but no definite ragged red change an increase in total mtDNA mirrored the increase in SDH staining (Fig. 7).

Studies of mtRNA showed proliferation of all species in RRF (probes 16S, CO2, ND5, L6). Non-ragged red COX deficient fibres also showed increased expression of H strand transcripts (probes 16S, CO2, ND5). These fibres were studied with image analysis; increases in all H-strand transcripts were in parallel, with preservation of the ratio between rRNA and H-strand mRNA species (probes 16S, ND5, CO2), demonstrated in Figs 7, 8. Histochemical features of patient G were similar to patient F but differed because of the higher proportion of histochemically abnormal fibres. Although only 5% of the fibres were COX negative, the majority of fibres were COX deficient. Mitochondrial DNA and mtRNA distribution followed a similar pattern to patient F.

Patient H. In the muscle biopsy from this patient with the bp 8344 mutation, RRF and COX negative fibres were uncommon, but COX deficient fibres were frequent. Most, but not all RRF were COX positive, showing subnormal central COX activity with relative preservation of activity in the subsarcolemmal region, differing from the pattern observed in patients F and G. Ragged red fibres showed a marked increase in mtDNA detected with any probe (16S, CO2, ND5, L6); mtRNA distribution followed the same pattern. A lesser increase in signal representing mtDNA or mtRNA was observed in non-RRF COX negative or COX deficient fibres.

The results of the *in situ* hybridization studies in all patients are summarized in Table 4.

DISCUSSION

The methods employed in this study differ in some respects to those used previously to study mtDNA and mtRNA tissue distribution in mitochondrial myopathies, and arguably offer an improvement. Use of oligonucleotides instead of larger probes obviates the need for tissue permeabilization procedures because of better tissue penetration (Tecott *et al.*, 1987), and thus preserves histological detail. The use of a probe (BRI) across the breakpoint region in one patient (B) allowed direct detection of DmtDNA and its transcripts, rather than indirect methods used previously (Mita *et al.*, 1989; Shoubridge *et al.*, 1990). Quantification of signal from *in situ* hybridization is difficult. The technique of comparison with adjacent fibres used here avoids the inherent inaccuracy of estimation

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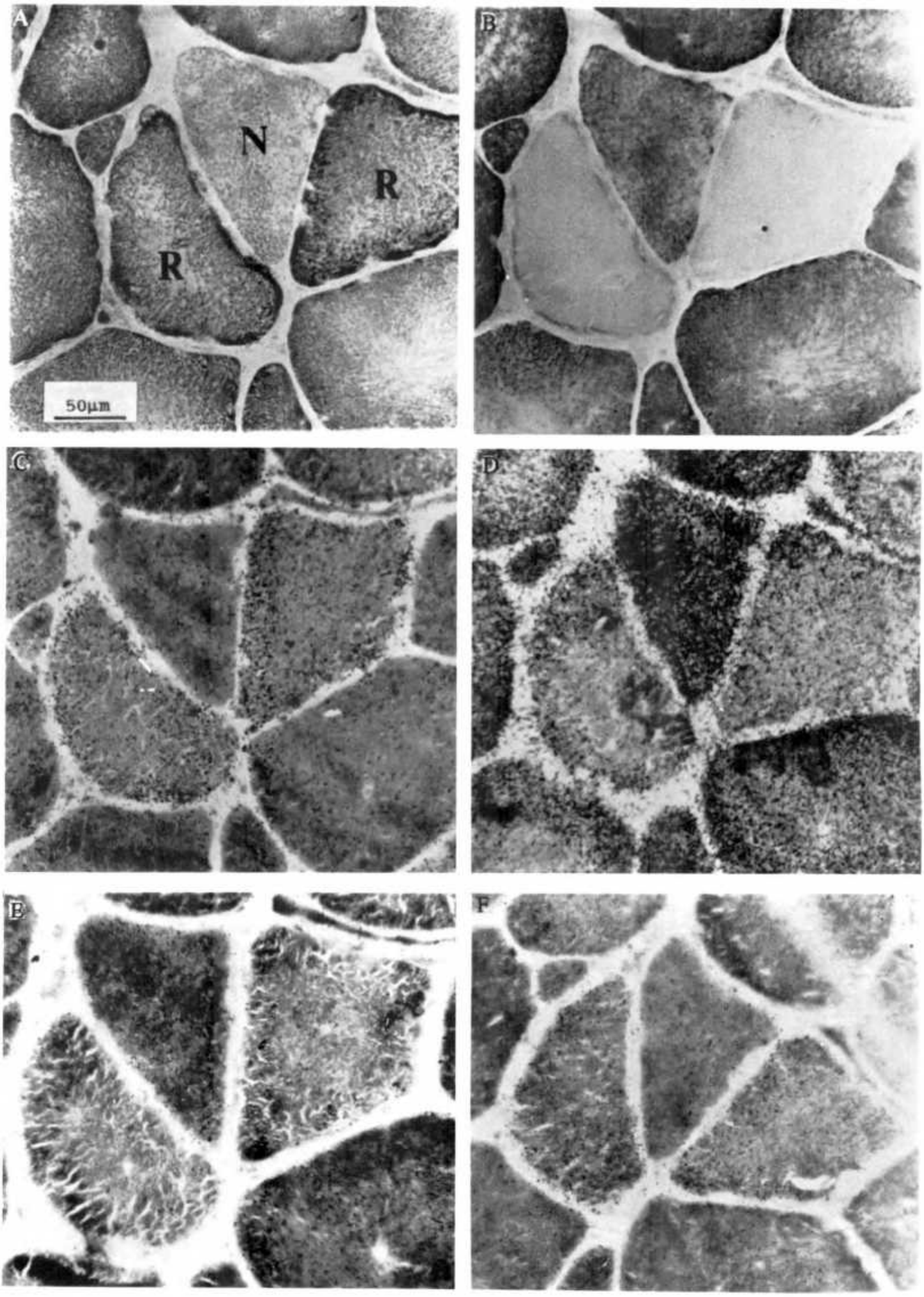

Fig. 6A–F

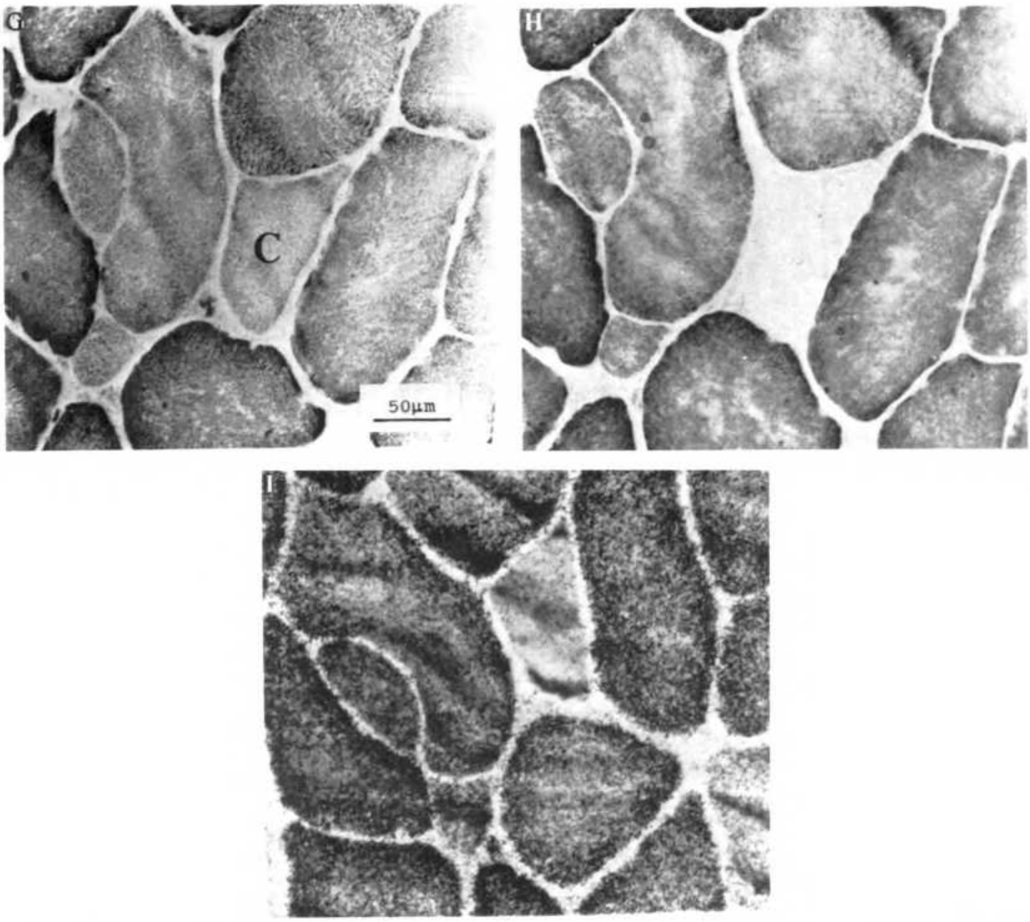

FIG. 6. Serial muscle sections from patient E. A, succinic dehydrogenase stain showing two RRF (R), both type 1 fibres, with a normal (N) type 2 fibre interposed. B, cytochrome oxidase (COX) stain showing that the two RRF fibres are COX deficient (*left*) and COX negative (*right*); other fibres stain normally. C, *in situ* hybridization detecting total mtDNA with probe CO2, demonstrating markedly increased signal within the RRF, particularly in the subsarcolemmal region, in contrast with adjacent normal fibres. D, *in situ* hybridization detecting 16S rRNA with probe 16S, with reduced signal in the two RRF compared with the adjacent normal fibres. E, *in situ* hybridization detecting all H-strand transcripts with probe CO2, demonstrating reduced signal with RRF. F, *in situ* hybridization with a probe detecting L-strand transcripts (L6) showing a distribution reflecting that of total mtDNA (Fig. 6C). G, succinic dehydrogenase (SDH) stain showing the presence of a fibre (C) with a mild decrease of SDH activity. H, cytochrome oxidase (COX) stain shows that this fibre is COX negative. Other COX negative fibres with depletion of mitochondria showed variable decreases in SDH activity often more marked than shown in Fig. 6G. I, *in situ* hybridization detecting 16S rRNA (probe 16S), markedly reduced in the fibre with depletion of mitochondria and absent COX activity.

of absolute values, and also minimizes errors arising from variation of emulsion thickness or in reaction conditions across the microscope slide. On detection of mtRNA, signal from normal type 1 fibres exceeded that of type 2 fibres approximately twofold. This is consistent with the different enzyme activities of the two fibre types (Johnson *et al.*, 1983; Reichmann, 1988).

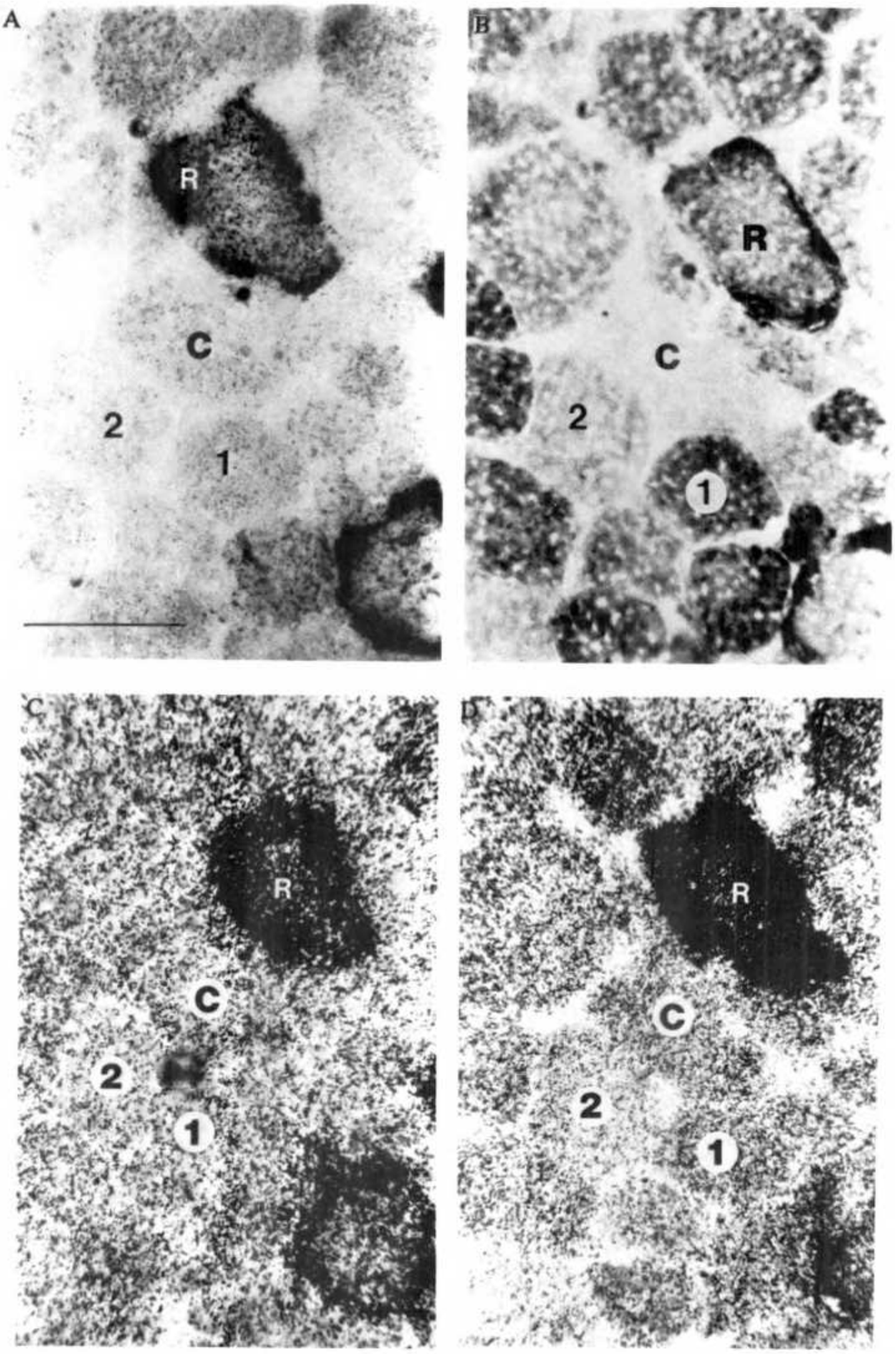

FIG. 7. Serial muscle sections from patient F. A, SDH stain demonstrating a RRF (R). Normal type 1 (1) and type 2 (2) fibres are labelled. A type 2 non-ragged red COX deficient fibre is labelled C. B, COX stain demonstrating subsarcolemmal regions of high COX activity within fibre R. Fibres 1 and 2 have COX activity appropriate to their fibre type, while fibre C is COX deficient. C, *in situ* hybridization with probe ND5 detecting ND5 mRNA. Fibre R has a high signal. Fibre 1 shows greater signal than fibre 2, corresponding to the higher mitochondrial content of type 1 fibres. The type 2 fibre C has higher signal than the normal type 2 fibre, with signal similar to that seen in the normal type 1 fibre. D, *in situ* hybridization with probe 16S detecting 16S rRNA, demonstrating high signal in fibre R. Fibre C shows increased signal to a level similar to that seen in a normal type 1 fibre. The histochemical defect is not accompanied by an absolute or relative quantitative deficiency of 16S rRNA.

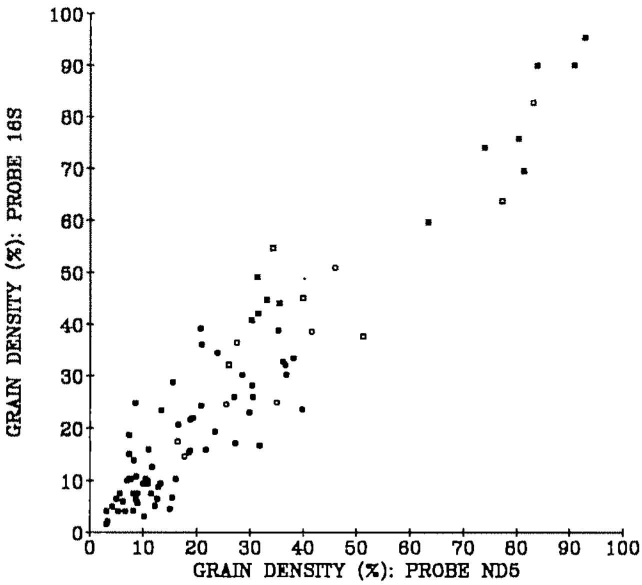

FIG. 8. Scattergram showing the results of image analysis of 88 fibres from the muscle biopsy of patient F following *in situ* hybridization with probes 16S and ND5. Results are expressed as a percentage of the area of each individual fibre occupied by silver grains. For this analysis 'COX deficient' fibres were defined as having no more than a trace of COX activity. Fibres with ragged red appearance on SDH staining are identified. The prevalence of the two species are clearly correlated; COX deficient fibres have a similar 16S rRNA/ND5 mRNA ratio to other fibres. Non-ragged red COX negative fibres usually show higher grain densities than non-ragged red COX positive fibres. ○, COX deficient fibres; ●, COX positive fibres; □, COX deficient RRF; ■, COX positive RRF.

Effect of a mtDNA deletion including the heavy strand promoter region

Patient E has a unique deletion of the smaller arc of mtDNA between O_H and O_L. It is flanked by an imperfect direct repeat, which supports the hypothesis that direct repeats are important in the pathogenesis of mtDNA deletions (Mita *et al.*, 1990). It extends to within 60 bp of the mitochondrial transcription factor 1 (mtTF1) binding site associated with the light-strand promoter (LSP) region (Topper and Clayton, 1989), and at its other limit deletes most of the reading frame of ND2. This contrasts with the three previous patients described with similar deletions. Moraes *et al.* (1991) described two patients with a deletion which affected only two bases of the mtTF1 binding site adjacent to HSP, some 30 bp upstream from our patient, and extended to the $tRNA^{Met}$ gene, not affecting the ND2 reading frame. The deletion of the patient described by Johns and Cornblath (1991) extended closer to the LSP but deleted less of the ND2 gene (Fig. 5).

Only four patients with deletions of the shorter mtDNA arc have been described, while more than 100 reported involved the larger arc. One explanation for this stems from the ninefold excess of 10–13 bp direct repeats in the larger arc (Johns and Cornblath, 1991). However, the 13 bp repeat within the HSP region has been involved in only two published deletions (Moraes *et al.*, 1991) in contrast to the 13 bp repeat flanking the 'common' deletion which accounted for 13 of 30 of our patients with deletions (Holt *et al.*, 1989*a*), illustrating that the site of repeats is important in deletion

TABLE 4. SUMMARY OF *IN SITU* HYBRIDIZATION AND HISTOCHEMICAL DATA

	Deletions							
	'Common' Patient A		*'Large' Patient B*		*'Small' Patients C and D*		*Deletion involving HSP Patient E*	
	RRF	*CONWOR*	*RRF*	*CONWOR*	*RRF*	*CONWOR*	*RRF*	*CONDM*
Probes detecting								
Total mtDNA	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑	↑↑↑	↓
Normal NmtDNA	≈	≈	≈	≈	≈	≈	≈	≈/↓
Deleted DmtDNA	n.d.	n.d.	↑↑↑	↑	n.d.	n.d.	n.d.	n.d.
Transcripts from both D and NmtDNA	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑	HS ↓ LS ↑↑↑	HS ↓ LS ↓
NmtDNA transcripts	↓	↓	↓	↓	↓	↓	↓	↓
DmtDNA transcripts	n.d.	n.d.	↑↑↑	↑/↑↑	n.d.	n.d.	n.d.	n.d.
COX activity	0/↓	¶	0/↓	¶	↓/0	¶	0/↓	¶

	Point mutations			
	Bp 3243 'MELAS' mutation Patients F and G		*Bp 8344 'MERRF' mutation Patient H*	
	RRF	*COX deficient fibres*	*RRF*	*CONWOR*
Probe detecting				
Total mtDNA	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑
16S rRNA transcripts	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑
ND5 mRNA transcripts	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑
COII mRNA transcripts	↑↑↑	↑/↑↑	↑↑↑	↑/↑↑
COX activity	↓–↑↑↑	↓	most ↓	¶

CONWOR = COX negative fibres without ragged red change; CONDM = COX negative fibres with depletion of mitochondria; HS = H-strand; LS = L-strand; n.d. = not done; n.a. = not applicable; ↑/↑↑/↑↑↑ = slight/moderate/marked increase compared with normal fibres; ≈ = no change over normal fibres; ↓ = decrease compared with normal fibres; 0 = absent; ¶ = COX negative by definition.

pathogenesis. Although the mechanism of deletion pathogenesis is unknown (Holt *et al.*, 1989*b*; Shoffner *et al.*, 1989; Mita *et al.*, 1990), secondary structure of the replicating genome and its interaction with replicatory and other proteins are likely to be important, perhaps giving rise to greater infidelity of replication of the larger arc of mtDNA.

Muscle histology was remarkable for the variation in fibre diameter, which was more marked than in any other biopsy seen with the same diagnosis at the authors' institution. It is possible that this finding is related to the rare site of the deletion, although the mechanism of this is unclear; no detailed histological findings were described in the other three patients with similar deletions.

The distribution of the total mtDNA in comparison with NmtDNA showed that the excess mtDNA within RRF was entirely of the deleted species, existing largely but not exclusively in subsarcolemmal aggregations of mitochondria. This population of DmtDNA within RRF appeared unable to produce H-strand transcripts, presumably because of deletion of the HSP region. However, the distribution of L-strand transcripts reflected that of total mtDNA signal within RRF. In contrast, L-strand transcripts in normal fibres and control muscle were detected only at low level, consistent with the rapid turnover of ND6 mRNA and resulting low copy number of steady state (Attardi *et al.*, 1990). Studies of promoter and transcription site sequences have suggested that promoter regions and mtTF1 may function bidirectionally (Chang *et al.*, 1986). Our data would suggest that the intact LSP region produced no significant transcription of the H-strand of deleted genomes. There was no apparent defect of L-strand transcription despite the lack of a possible contribution from the HSP complex, or the proximity of the deletion of the LSP complex. These data demonstrate, *in vivo*, the independent function of the LSP from that of the HSP complex.

The few CONDM fibres which were COX negative observed with mild to severe depletion of mitochondria differed from RRF. No excess mtDNA was found despite the presence of deleted mtDNA genomes as the major species. The fibres all showed profound depletion or absence of H-strand transcripts, more marked than in RRF. One possible cause of the absence of proliferation may result from failure to create an electrochemical potential across the inner mitochondrial membrane, since this potential is required for import of proteins into mitochondria. Deficiency of enzymes involved in replication or other functions may cause defective proliferation. A defect of mtDNA replication caused by the deletion affecting the LSP region, by virtue of its proximity, cannot be excluded, but this seems unlikely in view of the apparently efficient replication seen in RRF.

Distribution of NmtDNA and transcripts in patients with deletions

Detection of NmtDNA was more difficult than detection of its transcripts, requiring longer exposure and a corresponding increase in background grain density from non-specific signal. Conversely, 16S rRNA is easily detected, reflecting the relatively high steady state prevalence of rRNA species (Attardi *et al.*, 1990). For this reason, although NmtDNA was almost uniformly distributed among histochemically normal and abnormal fibres, depletion in the latter cannot be excluded. It is indeed probable, given the findings in relation to mtRNA.

Quantification of NmtDNA transcripts within RRF clearly showed that there was depletion of these species in all patients studied. Differences in median values obtained between the three patients with deletions of the larger arc of mtDNA are probably caused by differing reaction conditions producing a variable signal to noise ratio. Non-specific signal, non-linear response of the photographic emulsion and signal from adjacent fibres would all tend to obscure the extent of the depletion within RRF, and our estimations thus are likely to underestimate this rather than exaggerate it. Our data differ from those of Shoubridge *et al.* (1990), who found NmtDNA transcripts in RRF within the range of normal type 2 fibres. They did not quantify grain densities corresponding to transcripts of NmtDNA in their study, but their observation that levels within either fibre type were equivalent to levels in type 2 fibres suggest that depletion of NmtDNA transcripts occurred at least in type 1 fibres. Moraes *et al.* (1991) also studied the distribution of RNA in two patients with deletions affecting the HSP, but did not identify depletion of normal mtDNA transcripts in RRF. Mita *et al.* (1989) found depletion of NmtDNA in COX negative fibres but this was not quantified. These apparently conflicting data are most easily explained by the presence of non-specific signal obscuring focal depletions of NmtDNA and its transcripts (but not accumulations), as we observed in some sections prior to optimizing the technique described here.

Our findings indicate that focal depletion of NmtDNA is relevant to the pathogenesis of focal biochemical defects in mitochondrial myopathies. Depletion of NmtDNA transcripts is invariable within COX negative fibres and since this is accompanied by the presence of DmtDNA, it is not generally possible to separate the contributions of NmtDNA depletion and the presence of DmtDNA to reduced COX activity. However, two pieces of evidence suggest that the former is more important. First, patient E has a minority of fibres that show depletion both of mtDNA and H-strand transcripts, with preservation of numbers of L-strand transcripts. Thus, in this patient with an unusual deletion of mtDNA, it appears that depletion of mitochondrial rRNA species and some mRNA and tRNA species alone is sufficient to cause focal respiratory chain dysfunction. Second, in patient B, probe BRI detected the presence of DmtDNA transcripts within COX negative fibres without ragged red change, albeit at a much lower level than RRF. Focal COX deficiency invariably accompanied depletion of NmtDNA, but DmtDNA transcript accumulation and mitochondrial proliferation ranged from just discernible to very marked. Thus, in patients B and E, and probably in all patients with deletions, COX deficiency, NmtDNA transcript depletion and the presence of DmtDNA are closely associated. Mitochondrial proliferation and mtDNA overexpression are variable and likely to be a functionally ineffective response to the biochemical defect rather than a primary event.

Our studies using *in situ* hybridization reflect mtDNA and mtRNA density over cross-sectional area. In areas of mitochondrial proliferation a depeletion of NmtDNA density expressed per cross-sectional area implies a much greater depletion of NmtDNA per mitochondrion. Our results indicate a more than fourfold reduction of NmtDNA transcripts within type 1 RRF, while total transcripts are typically increased by 6.5fold. Estimates suggest that there are 2–10 copies of mtDNA per mitochondrion within mammalian or transformed human cells (Bogenhagen and Clayton, 1974; Robin and Wong, 1988). If this estimate can be extended to diseased muscle, a typical ratio greater than 26 between deleted and normal mtDNA transcripts within ragged red segments indicates that most mitochondria in ragged red segments contain only DmtDNA.

Longitudinal examination of individual muscle fibres in patients with mitochondrial myopathy shows ragged red segments and normal segments (Yamamoto and Nonaka, 1988; Shoubridge *et al.*, 1990); intervening junctional segments have increased SDH activity short of ragged red change. Viewed transversely a subpopulation of non-RRF in patients A and B are COX negative with variable increases in SDH activity, corresponding to areas within junctional segments. Our findings in patient B show DmtDNA is present in these fibre segments in lesser amounts than in RRF, with concomitant NmtDNA depletion. Thus a high DmtDNA/NmtDNA ratio in ragged red segments extends into the junctional segment, declining on transition to the normal segment. From the proportion of non-ragged red COX negative fibres in patients A and B, a substantial part of this junctional segment must be COX negative. COX activity returns, presumably at a threshold corresponding to a certain DmtDNA/NmtDNA ratio, and increases as the normal segment is approached. In patients C and D, an excess of RRF over COX negative fibres and rare COX negative non-RRF suggests that many ragged red and most junctional segments will generally have COX activity, albeit subnormal. The different histochemical characteristics of these patients requires explanation at the single fibre level.

Deletions of mtDNA create deficiencies of mRNA species derived from involved protein subunit reading frames, but all deletions hitherto described have also involved some tRNA genes. They would thus be expected to cause a translational defect involving all mtDNA encoded subunits. Although the distribution of different mtDNA species between mitochondria is unclear, coexistence of D and NmtDNA in the same mitochondrion could potentially ameliorate the translational defect, if NmtDNA supplied missing tRNAs (complementation). Evidence has been presented suggesting that complementation does not take place *in vitro* (Nakase *et al.*, 1990) or *in vivo* (Mita *et al.*, 1989) with the 'common' or larger deletions, but we have found evidence of complementation in some patients with deletions not involving COX subunits (Holt *et al.*, 1989*a*; Hammans *et al.*, 1992), including patients C and D. Near homoplasmy for DmtDNA in ragged red segments would effectively prevent translation of DmtDNA transcripts, irrespective of the site of the deletion, with consequent defects of all components of the respiratory chain containing mitochondrially encoded subunits. However, coexistence of D and NmtDNA in some organelles within junctional segments may provide sufficient tRNA numbers to allow complementation between genomes. These mitochondria should have greater COX activity if COX subunits are not deleted. At the single fibre level significant complementation in such organelles would explain the observation of conserved COX activity in RRF and junctional segments of patients C and D, contrasting with patients with deletions involving COX subunits. The absence of COX negative fibres in patient D implies, in this patient at least, that near or absolute homoplasmy for DmtDNA is not achieved across any fibre segment.

In summary, we have demonstrated that within muscle of patients with mtDNA deletions, DmtDNA and its transcripts are concentrated in histochemically abnormal fibre segments, with concomitant depletion of NmtDNA transcripts. The resulting deficiency of cytochrome oxidase activity appears to be partly dependent on the site of the deletion.

Effects of point mutations

Because defects of transcription termination at the 3′ end of the 16S rRNA have been demonstrated in the presence of the bp 3243 mutation *in vitro* (Hess *et al.*, 1991), the relative prevalence of different H-strand transcripts in normal and abnormal fibres was studied. The technique of *in situ* hybridization does not allow differentiation between the populations of MmtDNA and NmtDNA within individual fibres. Nevertheless, irrespective of the mtDNA content of different fibres, it appears that focal biochemical dysfunction is not a result of a relative reduction of rRNA species, since 16S rRNA appears to increase in parallel with mRNA species in histochemically abnormal fibres. Furthermore, if focal biochemical defects arise in fibres with a greater content of MmtDNA than histochemically normal fibres, as seems likely, it appears that the presence of the mutation does not disturb the ratio of H-strand transcribed rRNA/mRNA *in vivo*. It is possible that quantitatively defective termination is not apparent *in vivo* because of compensatory cellular mechanisms. These could act through post-transcriptional regulation of rRNA and mRNA concentrations, by up-regulation of mtTERM, or modulation of unknown transcriptional control systems. We have not excluded a qualitative effect of the mutation on the structure of the 3′ end of the 16S rRNA. The bp 3243 mutation may thus cause disease by one or more mechanisms. These include $\text{tRNA}^{\text{Leu(UUR)}}$ dysfunction, qualitative defects of transcription termination or secondary deleterious effects arising from compensation for quantitative defects of transcription termination.

One feature observed in muscle of patients with point mutations but not in patients with mtDNA deletions was the higher proportion of fibres with abnormal (usually subnormal) COX activity. This may be an indication of more diffuse distribution of mutant mtDNA in these patients in contrast to the more focal distribution of DmtDNA demonstrated directly in patient B and indirectly in patients A, C, D, E.

The results of *in situ* hybridization studies in patients F, G, H with two different point mutations of mtDNA were broadly similar, but there were differences in the histochemical features associated with the observed excess of mtDNA and its transcripts in RRF, depending on the mutation. In patients F and G (bp 3243 mutation), RRF often, but not always, showed patchy hyperdense COX staining. Although many COX deficient fibres were noted, relatively few were COX negative. In contrast, patient H (bp 8344 mutation) generally showed subnormal COX staining within RRF, and relatively more COX negative fibres were observed.

Differences in COX activity of RRF between patients with different genetic defects have not previously been described. Further patients have been studied histochemically to verify these observations. Adjacent muscle sections stained for COX and SDH were studies; patients had either the bp 8344 (n = 3) or bp 3243 (n = 5) mutation (Hammans *et al.*, 1991). Though the biopsies contained differing numbers of RRF, in the presence of the bp 8344 mutation RRF generally showed subnormal COX activity. With the bp 3243 mutation, RRF showed variable COX activity, including some with characteristically patchy hyperdense staining.

Correlating results of biochemical studies of pooled mitochondria from patients with different genetic abnormalities does not always give consistent results (Table 5). However, it appears that the MELAS syndrome (and probably the bp 3243 mutation) is commonly associated with complex I deficiency. Deletions of mtDNA not involving COX subunits may also be associated with complex I deficiency on polarography (Hammans *et al.* 1992). With the bp 8344 mutation and deletions involving COX and other subunits there is often a diffuse respiratory chain defect including complex IV. At the single fibre

TABLE 5. SUMMARY OF BIOCHEMICAL FINDINGS ASSOCIATED WITH GENETIC DEFECTS

Genetic defect or clinical syndrome	*Reported biochemical defect*	*References*
Large heteroplasmic deletions	Complex I	Holt *et al.*, 1989
	Complex I–III	Gerbitz *et al.*, 1990
	Complex I–IV or no defect	Moraes *et al.*, 1989 and Goto *et al.*, 1990*a*
Deletions sparing COX subunits	Complex I	Holt *et al.*, 1989 and Hammans *et al.*, 1992
MERRF	Complex IV	Lombes *et al.*, 1989
	Complex III,	Berkovic *et al.*, 1989, 1991*
	Complex III and IV	
	Complex I and IV	Wallace *et al.*, 1988*
	Complex I, III and IV	Bindoff *et al.*, 1991**
MELAS or bp 3243 mutation	Complex I	Koga *et al.*, 1988
	Complex I and IV	Yoneda *et al.*, 1989
	Complex I 6/10 patients	Unpublished results***
	Complex I–III 3/10 patients	
	Normal 3/10 patients	

* = bp 8344 mutation present; ** = bp 8344 mutation not present; *** J. M. Cooper, unpublished polarographic data from 12 patients with the bp 3243 mutation, includes patients F and G; only 5/12 have clinical syndrome of MELAS.

level, COX deficiency invariably accompanies DmtDNA in RRF if the deletion involves COX subunits, suggesting that COX deficiency is a direct biochemical effect of the deletion. A tendency towards preservation of the COX activity of RRF in the presence of the bp 3243 mutation, and to a lesser extent with the bp 8344 mutation and with deletions not involving COX subunits genes, suggests that COX deficiency is not the biochemical lesion evoking the response of mitochondrial proliferation. Polarographic data suggest that complex I deficiency may be the primary defect in some cases, while COX activity is increased or decreased as a lesser or secondary effect.

The mitochondrial encephalomyopathies exhibit clinical, biochemical and genetic diversity. Although they share the morphological hallmark of the RRF, usually together with histochemical evidence of focal COX deficiency, it is clear that the molecular pathology of these common features is heterogeneous and complex.

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THE LEFT MEDIAL TEMPORAL REGION AND SCHIZOPHRENIA

A PET STUDY

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SUMMARY

Regional cerebral blood flow (rCBF) was measured in 30 schizophrenic patients with severe, persistent and stable symptoms using positron emission tomography (PET). Directed and non-directed correlational analysis of the relationship between psychopathology and rCBF was used to identify brain structures implicated in three behavioural subsyndromes of schizophrenia. Psychopathology and neurophysiology (rCBF) exhibited high correlations in the left medial temporal region, mesencephalic, thalamic and left striatal structures. The highest correlation was in the left parahippocampal region. A canonical analysis of the same data highlighted the left parahippocampal region and left striatum (globus pallidus) as sites which linked the behavioural subsyndromes in terms of shared rCBF correlates.

Increasing severity of psychopathology was associated with increased rCBF in these regions. Disinhibition of left medial temporal lobe activity mediated by fronto-limbic connections is a possible explanation for these findings; however, the prefrontal component appears to be critically dependent on the behavioural subsyndrome.

INTRODUCTION

In an early mechanistic account of schizophrenia, Bleuler (1911) distinguished between primary and secondary processes. This distinction has been variously interpreted (Raskin, 1975). Primary processes emphasize a disintegration or absence of mental activities with loss or fractionation of normal behaviour. Lehmann (1967) considers that Bleuler described three primary symptoms—disturbances of associations, affectivity and initiative—and refers to the four 'A's: associations, affect, autism and ambivalence. Secondary symptoms such as hallucinations, delusions, blocking and catatonic behaviour were considered to be responses to primary symptoms.

Recently, two different subdivisions of schizophrenia have enjoyed success. The first of these is a categorical distinction between paranoid and non-paranoid schizophrenia. For example, Tsuang and Winokur (1974) distinguish between hebephrenic (with inappropriate or flat affect and formal thought disorder) and paranoid patients (characterized by well-organized delusions and hallucinations). A positive-negative dichotomy has been proposed partly on the basis of the differential response of symptoms

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to drug treatment (Crow, 1980). Negative symptoms include poverty of speech, reduced spontaneous movement, poverty of affect and withdrawal. Positive symptoms include thought disorder, hallucinations and delusions.

Disorders of thought form and inappropriate affect do not sit comfortably in these categorical or two-dimensional schemes. Some feel they represent positive symptoms (Crow, 1980) yet they are more closely allied to non-paranoid or Bleulerian primary symptoms. Thought disorder and incoherent speech are primary symptoms and reflect 'loosening of associations'. Bleuler (1911) initially considered inappropriate affect was a secondary symptom but later (Bleuler, 1930) placed it in the primary class describing '. . . the tendency of the feelings to work independently of each other . . . evident, for instance, in the ambivalence, in inadequate affective reactions, simultaneously laughing and crying'.

The issue concerning thought disorder and inappropriate affect has been partially resolved by controlling for symptom persistence in studies of patients with severe and stable symptoms (Liddle, 1987). Factor analysis has demonstrated three independent groupings; psychomotor poverty, characterized by poverty of speech, affect and movement; reality distortion, comprising hallucinations and delusions; and disorganization syndrome, which includes formal thought disorder and inappropriate affect. This three-dimensional structure has been replicated (Liddle and Barnes, 1990) and similar findings have been reported (Bilder *et al.*, 1985; Mortimer *et al.*, 1990; Arndt *et al.*, 1991).

Each of these subsyndromes can coexist independently in the same individual. This suggests that three or more distinct *functional* brain systems are affected. The hypothesis that each subsyndrome is associated with a distinct and unique profile of regional cerebral activity was tested and confirmed by identifying brain regions whose regional cerebral blood flow (rCBF) correlated with severity scores for each subsyndrome (Liddle *et al.*, 1990). These regions corresponded to three, large non-overlapping, distributed brain systems that were confined to (para)limbic and association cortices and related subcortical structures. The cardinal features of these distributed profiles can be summarized as follows. (i) Psychomotor poverty correlated negatively with rCBF in the prefrontal and left parietal association cortices, with positive correlations in the caudate nuclei. (ii) Reality distortion was associated with increased rCBF in the left parahippocampal region, left ventral striatum and in left prefronto-temporal regions. Negative correlations with severity or reality distortion were limited to the right hemisphere, notably in the posterior cingulate and caudate. (iii) Disorganization syndrome was characterized by marked positive correlations in the anterior cingulate and negative correlations in the right temporo-insular-prefrontal cortices. These results support the notion that the neurophysiological correlates of schizophrenic symptomatology are distributed over extensive brain systems (Frith and Done, 1988). Furthermore, these systems are comprised of limbic, paralimbic and association cortex and subcortical structures which project to, or receive projections from, these cortices. Many of these findings relate the behavioural deficits of the syndromes to known functional anatomy and established neuroanatomical connections. *See* Liddle *et al.* (1992) for a full description.

Because function is anatomically distributed (Mesulam, 1990) it is probable that *functional* brain systems associated with behavioural subsyndromes of schizophrenia have common *neuroanatomical* components. This is important because pathophysiological mechanisms act at a level (of description) that is anatomical not functional. The objective of the present analysis was to identify these common, or central, neuroanatomical components. Identifying a central abnormality is complicated because a region whose neurophysiology is substantially correlated with several independent subsyndromes cannot evidence a high correlation with any one in isolation. In a sense a shared neurophysiological abnormality will be pulled in several independent directions. We addressed this issue by (i) examining the correlations between rCBF and a measure of psychopathology which received equal contributions from all three subsyndromes and (ii) a canonical analysis of the neurophysiological and psychopathology data. A canonical analysis was used to examine how all three subsyndromes, or pairs of subsyndromes were linked through common brain regions. We predicted that, because function is distributed, each independent behavioural subsyndrome would share some central neurophysiological dysfunction. This central abnormality would be revealed by a canonical vector that shared positive loadings on all three subsyndromes, or would be found in all canonical vectors linking pairs of subsyndromes.

This analysis is exploratory and led by the data.

METHODS

Patients

Thirty chronic schizophrenic patients (DSMIII-R: American Psychiatric Association, 1987) consented to participate. Patients were recruited whilst undergoing psychiatric rehabilitation on an in-patient basis. The cohort studied comprised about a third of the total population available for selection of patients. Those included in the study were representative of this chronic but stable group. The selection criteria emphasized severe, persistent and stable symptoms. All patients were under the age of 55 yrs with no other known neurological history or history of substance abuse according to SADS-L (Endicott and Spitzer, 1978). Mental state was assessed at monthly intervals using the Manchester Scale (Krawiecka *et al.*, 1977). Patients were scanned during a 3 mth period in which the greatest change in any item was one. During this period there was no change in medication. Permission to perform this study was obtained from the local ethical committee and the Administration of Radioactive Substances Advisory Committee. The patients (25 males and five females) were aged between 21 and 54 yrs (mean = 36.3 yrs, SD = 9.6 yrs). One was an inconsistent left-hander and two were consistent left-handers. Mean age of onset (age at first psychotic episode according to SADS-L) was 21.7 yrs (SD = 5.2 yrs). Mean duration of illness was 14.6 yrs (SD = 8.44 yrs). Mean daily dose of chlorpromazine equivalents was 1465 mg equiv. (SD = 1011 mg equiv.).

Symptoms at the time of scanning (within a 2 wk period following the scan) were assessed, using Section 2 of the Comprehensive Assessment of Symptoms and History (CASH; Andreasen, 1986).

Regional cerebral blood flow measurements

Images of regional cerebral blood flow (rCBF) were obtained using the steady state oxygen inhalation technique (Frackowiak *et al.*, 1980). Scans were performed with a PET scanner (CTI model 931-08/12, Knoxville, USA) whose physical characteristics have been described (Spinks *et al.*, 1988). Scans were reconstructed using a Hanning filter with a cut-off frequency of 0.5 giving a transaxial resolution of 8.5 mm. A thermally moulded headrest supported the back of the head. The transaxial plane of the scanner was aligned parallel to the orbitomeatal line. Before starting, each patient was told to close his or her eyes and asked if he or she was comfortable. No additional instructions were given. Scanning was performed in a dimly lit and quiet room.

Stereotactic normalization

The 15 original scan slices (6.75 mm interplane distance) were interpolated to 43 planes to render the voxels approximately cubic. The intercommissural line was identified directly from the PET image (Friston *et al.*, 1989) and the images transformed into a standard stereotactic space (Fox *et al.*, 1985; Talairach and Tournoux, 1988; Friston *et al.*, 1991*a*). In this space one voxel represents 2×2×4 mm in the x, y and z dimensions according to the atlas of Talairach and Tournoux (1988). This approach allows registration of cortical structures to within a millimetre or so and subcortical structures to within about 5 mm. The precision of the stereotaxis is consequently in the order of millimetres (Friston *et al.*, 1991*a*). Each image was smoothed with a Gaussian filter 10 pixels wide to account for normal variability in gyral anatomy.

Comparison of behavioural and rCBF data

Two approaches were taken. A directed analysis which correlated a single psychopathology severity score with rCBF and a non-directed, data led, canonical analysis of both behavioural and rCBF data sets. Canonical analysis (Hope, 1968) is similar to principal component (PC) analysis in that both identify patterns or profiles which account for the most variance in the data. Canonical analysis is an extension which preserves the distinction between one set (psychopathology) and another set (rCBF) of scores. Canonical analysis was used to find the patterns of profiles of brain activity which were highly correlated with a combination of behavioural scores.

The null hypothesis, that each behavioural syndrome is associated with dysfunction in a separate, no-overlapping brain system, suggests three such profiles would be expected, each associated with one, and only one subsyndrome. If function is distributed across anatomical brain systems there should be considerable overlap, a central neurophysiological abnormality, i.e. some profiles will correspond to the commonalities shared by two or more subsyndromes. To reduce the sample size: dimensionality ratio both the symptom and rCBF scores were reduced in number. The symptom assessment scores were subject to factor analysis and the factor scores on the first three factors used as subsyndrome scores. The rCBF data were analysed only at pixels in which a significant ($P < 0.05$) amount of variance could be predicted by the subsyndrome scores. Regional cerebral blood flow in the remaining parts of the brain could not be predicted by psychopathology in this data set and were therefore not subject to further analysis. Because we studied only 30 subjects the number of rCBF measurements entered into the correlational analysis was reduced to 10 by using the first 10 PC scores following a PC analysis of the rCBF data. Ten was an arbitrary choice ensuring the number of subjects was substantially more than the number of observations (10 rCBF PC scores and three subsyndrome scores). In our experience the first 10 PCs usually account for more than 90% of the total variance in rCBF data. This was confirmed *post hoc*.

Factor analysis of symptom scores

The symptom ratings entered into the factor analysis were scores for delusions, hallucinations, positive formal thought disorder, poverty of speech, affective flattening, poverty of content of speech, poverty of spontaneous movement and inappropriate affect, obtained by summing the appropriate item scores in the relevant subscales.

Factor analysis was performed using the Statistical Package for Social Sciences (Nie *et al.*, 1975). Principal component extraction of factors was followed by varimax rotation. Factor subsyndrome scores were calculated by the regression method.

Regional cerebral blood flow data

All rCBF pixel values were adjusted, using linear regression, to remove differences in whole brain global CBF. This adjustment has been validated in removing the effect of global variance within and between subjects (Friston *et al.*, 1990). It was confirmed that global CBF did not correlate with any subsyndrome score or their sum. The amount of variance in rCBF that could be predicted by the three subsyndrome scores was assessed with the F ratio using multiple regression with subsyndrome scores as predictor and rCBF as predicted varia bles (Hope, 1968). Only pixels significant at $P < 0.05$ were analysed further. The significance of the resulting profile was assessed by comparing the observed and expected number of significant pixels (Friston *et al.*, 1991*b*).

Correlation of rCBF and psychopathology score

The sum of the three subsyndrome scores was correlated with adjusted rCBF at each pixel. This is equivalent to a second order correlation between rCBF and psychopathology partialling out the effect of global CBF. The profile of correlation coefficients over the brain was presented as a statistical parametric map (SPM). Brain regions with a high coefficient correspond to structures whose rCBF shares a substantial amount of variance with all three subsyndromes.

Canonical analysis

The three subsyndrome scores were entered into a canonical analysis with the first 10 rCBF PC scores derived from the normalized rCBF data matrix and eigenvectors of the correlation matrix. The significance of the canonical roots was assessed using the χ^2 statistic (Hope, 1968). The canonical vectors corresponding to subsyndrome scores and roots were presented in tabular form. The profiles of rCBF corresponding to the rCBF canonical vectors were displayed as images (SPMs). These SPMs were the sum of the 10 PCs, loadings for each pixel, weighted according to the canonical vectors, loadings for each components. This is simply a way of presenting the rCBF canonical vectors in image format.

RESULTS

Factor analysis of symptom scores

The factor analysis of symptom scores was in accord with previous findings. Three factors had eigenvalues greater than unity. These factors accounted for 75.9% of the variance in symptom scores. The rotated factor matrix is shown in Table 1. The first factor corresponds to psychomotor poverty and is characterized by poverty of speech, flattening of affect and poverty of movement. Factor 2 corresponds to disorganization syndrome with formal thought disorder, poverty of content of speech and inappropriate affect. The third factor corresponds to reality distortion characterized by delusions and hallucinations.

There were no significant ($P < 0.05$) correlations between the three subsyndrome scores and age, duration of illness, educational attainment or dose of medication.

Regional cerebral blood flow data

Global activity did not correlate with any of the three subsyndrome scores of their sum. The number of pixels in which rCBF could be predicted by subsyndrome scores was significantly greater than chance expectation ($\chi^2 = 70.36$; d.f. 1; $P < 0.00001$).

TABLE 1. FACTOR ANALYSIS OF SYMPTOM SCORES

	Factor 1 Psychomotor poverty	*Factor 2 Disorganization syndrome*	*Factor 3 Reality distortion*
Poverty of speech	0.88		
Flattening of affect	0.86		
Decreased movement	0.80		
Inappropriate affect		0.87	
Formal thought disorder		0.75	
Poverty of content of speech		0.75	
Delusions			0.90
Hallucinations			0.83

Rotated factor matrix of loadings on all three factors with eigenvalues greater than unity following a factor analysis of symptom scores derived from Section 2 of CASH (Andreasen, 1986). Loadings of less than 0.5 have been omitted for clarity. The corresponding subsyndrome is given for each factor. The factor scores associated with each of the three factors were entered into a canonical analysis with 10 rCBF PC scores (reproduced with permission from Liddle *et al.*, 1992).

The corresponding brain regions were distributed but universally limited to association or (para)limbic cortices and related subcortical structures.

Correlation between rCBF and psychopathology score

The profile of positive correlation coefficients is seen in Fig. 1A. The most remarkable feature of this profile is the positive correlation between left parahippocampal rCBF and the sum of the three subsyndrome scores. This represents the highest correlation ($\varrho = 0.613$, d.f. 28) over the entire brain. This location is at the centre of a region which extends down the parahippocampal gyrus [−12 mm, −30 mm, 4 mm, BA 30 according to the atlas of Talairach and Tournoux (1988), $\varrho = 0.56$] into entorrhinal cortex (−14 mm, −26 mm, −8 mm, BA 28, $\varrho = 0.53$) and medially to incorporate the left midbrain, thalamic (0 mm, −22 mm, 4 mm, dorsomedial nuclei, $\varrho = 0.48$) and left striatal structures (−10 mm, −2 mm, 8 mm, globus pallidus, $\varrho = 0.50$). Although these coordinates seem very precise it should be remembered that the data

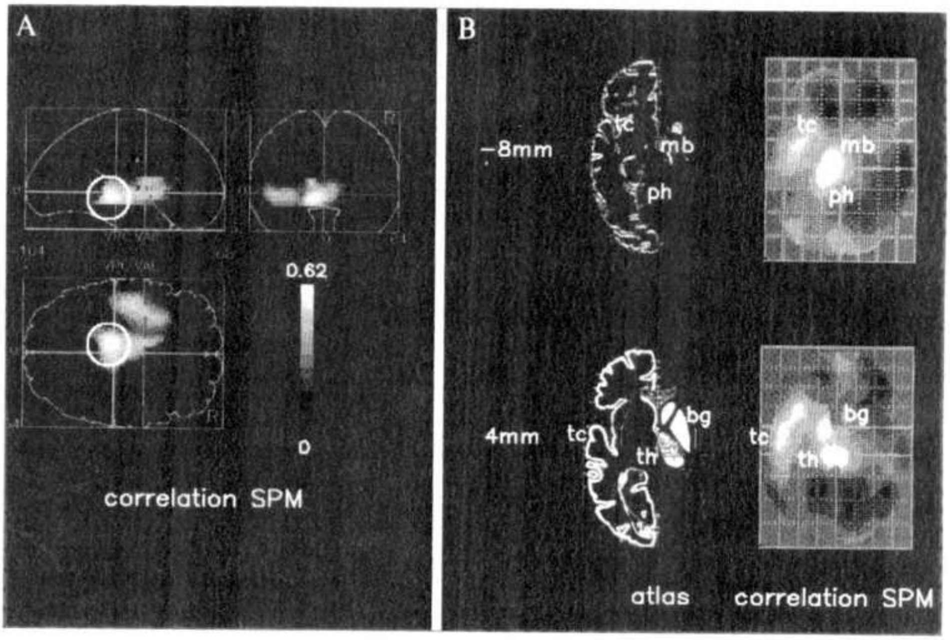

FIG. 1. Statistical parametric maps (SPMs) of correlation coefficient relating overall psychopathology with rCBF. A, SPM of the positive correlations between the sum of all three subsyndrome scores and adjusted rCBF. The scale is given in the bar insert. The most notable feature of this profile is the marked correlations of the left parahippocampal region. The highest correlation in this area was 0.613. The left parahippocampal region has been circled for reference. The three orthogonal views of the brain display the highest pixel value along the line of view. The three views correspond to the back of the brain (*right*) the right side (*left top*) and the top of the brain (*lower left*). B, The same data depicted in A displayed for two transverse sections at, 8 mm (*top*) and 4 mm (*bottom*) relative to the AC-PC line. The SPMs are of the correlation coefficient between the sum of all subsyndrome scores and rCBF. The regions highlighted are significant ($\varrho > 0.361$, $P < 0.05$, d.f. 28, no correction for multiple correlations) and illustrate the topography of the temporal and subcortical brain regions implicated by this analysis. ph, parahippocampal region; mb, midbrain at the level of the substantia nigra and coextensive ventral tegmentum; tc, temporal cortex (temporo-polar, medial superior temporal and contiguous insular cortex; th, medial thalamus; bg, basal ganglia, medial aspects of lentiform nucleus centred on globus pallidus.

represent a weighted average of rCBF in a spherical domain of 20 mm diameter. Additional observations on this profile include a second region of positive correlations in the temporal lobe centred on superior and temporo-polar region and contiguous insula. Negative correlations were less significant and were limited to the right insular cortex, related frontal operculum and bilateral superior parietal association cortex (BA 39/40). Figure 1B shows the same data for two transverse sections at −8 mm and 4 mm relative to the AC-PC plane. Pixels at which ϱ was significant ($\varrho > 0.361, P < 0.05$, d.f. 28 one-tailed, no correction for multiple comparisons) are highlighted.

Canonical analysis

The first 10 PCs of the rCBF data accounted for 89.41% of the total variance. Table 2 summarizes the results of the canonical analysis. Of the three canonical vectors, the first two accounted for 75.96% of total variance. Significance of the first and subsequent roots was $P < 0.005$ ($\chi^2 = 55.6$, d.f. 30). Table 1B shows that the first and second canonical vectors accounted for similar amounts of variance (canonical roots). The segregation of syndrome scores was interesting. The first canonical vector received no contribution from psychomotor poverty and reflects the rCBF effects shared by reality distortion and disorganization syndrome. Conversely, the second canonical root receives little contribution from reality distortion and predominantly reflects psychomotor poverty with some contribution from disorganization syndrome. The key feature of the canonical vectors is that disorganization syndrome is related through common rCBF profiles to both reality distortion and psychomotor poverty but reality distortion and psychomotor poverty were themselves largely unrelated. The corresponding canonical rCBF profiles (positive loadings) are seen in Fig. 2A.

TABLE 2. CANONICAL ANALYSIS OF SUBSYNDROME AND rCBF SCORES

	Canonical vectors		
	First	*Second*	*Third*
Psychomotor poverty	−0.08	1.33	−0.23
Disorganization syndrome	0.74	0.39	0.44
Reality distortion	0.66	−0.26	−0.52
Canonical roots	0.70	0.60	0.23

Canonical vectors for subsyndrome scores. The significance of the corresponding canonical roots was assessed using the χ^2 statistic. The key features of these results are that the first two canonical vectors account for most of the variance (84.63%). The first canonical vector shares an equal amount of variance with reality distortion and disorganization syndrome but none with psychomotor poverty.

The first canonical profile had high positive loadings on the left superior temporal and insular cortex, on the left parahippocampal gyrus, left midbrain, striatum and in the right anterior cingulate. Negative loadings are seen exclusively in the right hemisphere in the insular cortex, in the right head of caudate, right parietal association cortex and right posterior cingulate. This profile is characterized by a left-right asymmetry (with the exception of the right anterior cingulate). The rCBF profile associated with the second canonical vector showed high positive loadings in the left parahippocampal gyrus and

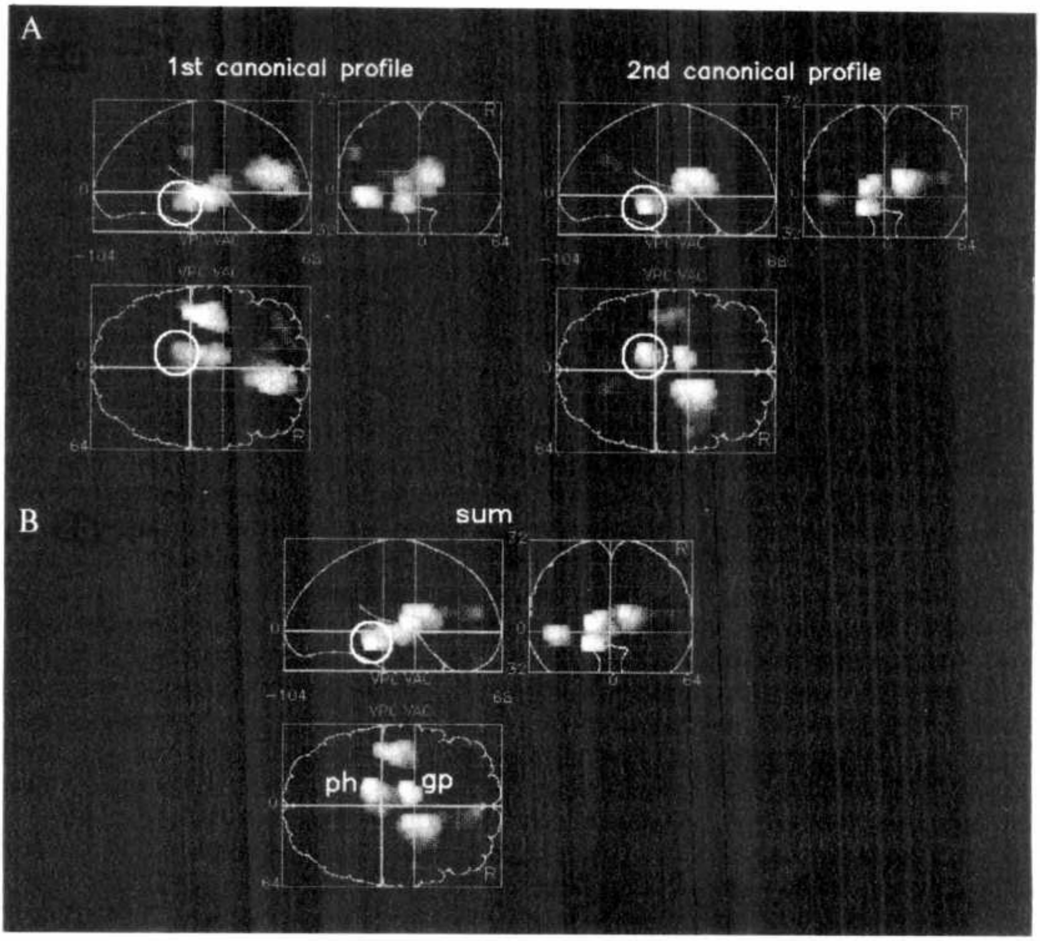

FIG. 2. SPMs representing the loadings on the first two canonical vectors following a canonical analysis of the psychopathology and rCBF data. A, SPMs in the same format as for Fig. 1A but depicting positive loadings on the first two canonical vectors following the canonical analysis of subsyndrome scores and rCBF PC scores. These SPMs are weighted averages of the 10 PCs entered into the canonical analysis. The weighting was according to the two canonical vectors. The main features of the profiles and the neuroanatomical regions highlighted are described in the main text. B, voxels at which loadings were positive for both canonical vectors. The pixel values are the sum of the loadings from the first and second vectors. ph, parahippocampal region; gp, globus pallidus. The scale in these SPMs is arbitrary. Each SPM was normalized to the greatest pixel value.

bilaterally in the anterior thalamic nuclei and striatum. Negative loadings (not shown) are seen in the left anterior and dorsolateral prefrontal cortex (DLPFC BA 10,9 and 46) and parietal association cortex. This profile represents a cortico-subcortical imbalance. The first two canonical profiles reflect the neurophysiological commonalities that link reality distortion and disorganization, and disorganization and psychomotor poverty, respectively. The third profile is that which distinguishes disorganization from the other two (not described).

Comparison of the two rCBR canonical profiles which link subsyndrome pairs (first and second) demonstrated that the left parahippocampal region is common to both. This point is illustrated in Fig. 2B, which shows all pixels with positive loadings on the first and second canonical vectors. The pixel values are the sum of the loadings on these two canonical vectors. The maximum value was found in the parahippocampal region (−16 mm, −32 mm, −8 mm, BA 35). This region extends from −8 mm to 0 mm relative to the AP-PC line and extends posteriorly and dorsally from medial temporal lobe, BA 28 (entorrhinal cortex) to the posterior extent of BA 30 (parahippocampal gyrus). The second largest value was found in the left globus pallidus, a result that might have been predicted by Early *et al.* (1987). The other regions highlighted in Fig. 2B are the right head of caudate and left temporo-insular cortex. Given that the two canonical profiles represent brain systems jointly implicated in the two subsyndrome pairs the left posterior parahippocampal region is a likely site of the central neurophysiological abnormality found in schizophrenia. Other candidates include the striatum (notably left globus pallidus) and left temporal cortex.

DISCUSSION

Using a directed and non-directed correlation analysis of severe, persistent symptoms and rCBF in chronic schizophrenia we have demonstrated that the left parahippocampal region is an area whose neurophysiology may be central in schizophrenia irrespective of behavioural subsyndrome.

A canonical analysis of psychopathology and rCBF data has been presented. This somewhat complicated analysis was used to illustrate two fundamental points. First, that the left parahippocampal region was common to the patterns of rCBF that link the physiological and behavioural data. Secondly, there is no simple segregation of independent behavioural syndromes and patterns of brain activity. This allows us to infer that function is anatomically distributed in so far as those functions represent cognitive processes impaired in schizophrenia. A more detailed consideration of the canonical results suggest that thought disorder and inappropriate affect (disorganization syndrome) are related to positive symptoms of reality distortion, and to a lesser extent the negative symptoms of psychomotor poverty. This relationship is implicated through the neuroanatomical systems. This is consistent with the notion that the symptoms of disorganization syndrome are positive, in that they do not represent the absence of normal behaviour. The view that disorganization and psychomotor poverty symptoms are of the same non-paranoid or primary 'Bleulerian' class is also supported by the second canonical profile. In terms of cerebral activity (rCBF) there appear to be two important relationships between the three behavioural syndromes. These relationships (reality distortion-disorganization and disorganization-psychomotor poverty) have parallels at the level of symptoms. Liddle and Barnes (1990) have discussed the overlap between reality distortion and disorganization syndrome in terms of thought disorder.

This was a within group correlational study. We do not consider a suitable control group exists for categorical comparisons in chronic schizophrenia. However, the main reason for adopting correlational statistics is the (assumed) dimensional structure of schizophrenic subsyndromes. If the independent coexistence of several symptom groupings in a single individual is to be modelled then dimensional-correlational approaches are required. This approach obviates the need for a 'control' group but it should be pointed out that if a single neurophysiological abnormality is common to all schizophrenias then correlational analysis relies on the degree of this abnormality being correlated with psychopathology. Early *et al.* (1987) have demonstrated left globus pallidal hyperactivity in patients with schizophrenia who have never been medicated. This observation was categorical and is supported by the present correlational findings (correlation between overall severity and rCBF in the left globus pallidus −10 mm, −2 mm, 8 mm, $\varrho = 0.50$, d.f. 28). This region is seen clearly in the second canonical profile (Fig. 2B).

Hypofrontality (Ingvar and Franzén 1974) has been one of the more robust categorical findings in functional imaging studies of schizophrenia. However, even with regard to hypofrontality there have been divergent findings. DeLisi *et al.* (1985*a*) compared chronic schizophrenic and control subjects. The patients were free of medication for at least 2 wks before the study. Patients had significantly lower anterior-posterior gradients (hypofrontality) than the control group. Cerebral atrophy, as determined by computerized tomography (CT) was not correlated with this aberrant pattern. Gur *et al.* (1987*a*,*b*) have described abnormalities of cortico-subcortical metabolic gradients in schizophrenic patients but found no evidence for hypofrontality. Finally, Szechtman *et al.* (1988) examined whether the duration of treatment influenced the regional distribution of metabolism in patients with schizophrenia. The schizophrenic group was dichotomized according to treatment duration. Both groups evidenced *hyper*frontality when compared with controls. This was less evident in the group with the longest exposure to medication. One explanation for these inconsistencies may be the relative amounts of each behavioural subsyndrome the patient groups expressed. One can find a partial resolution in the present findings, in that both *hyper*frontality (anterior cingulate in reality distortion and disorganization syndrome, first canonical profile) and *hypo*frontality (DLPFC in psychomotor poverty and disorganization syndrome, second canonical profile) can coexist in the same patient, this coexistence being apparent at both a behavioural and neurophysiological level. DeLisi *et al.* (1985*b*) reported that the only significant correlations between relative hypofrontality and symptom ratings were for emotional withdrawal, disorientation, distractability and helplessness/hopelessness—symptoms found in the psychomotor poverty and disorganization syndromes. The negative loadings in the prefrontal cortex on the second canonical profile are consistent with this observation.

Because the brain is massively interconnected abnormal rCBF in a given region may derive from a primary, local pathophysiology or be a secondary consequence of remote abnormalities mediated by interconnections.

Neuroanatomical distribution and interconnections

The two canonical profiles implicated the following neuroanatomical components: the left entorrhinal and parahippocampal gyrus, left subcortical structures (midbrain, ventral striatum, thalamus and striatum), the anterior and posterior cingulate, the left DLPFC, the insular cortices (with right opercular), superior parietal cortices and superior temporal cortices. Goldman-Rakic (1987) discusses the neuroanatomical substrate of behavioural regulation by representational memory and introduces a 'Quadripartite Neural Network: Parietal-temporal-cingulate-prefrontal circuit'. She argues for an involvement of hippocampal-prefrontal connections in mnemonic processing by implicating these connections and parietal-prefrontal connections in a larger system. Injection of two distinguishable tracers, one into the principal sulcus and the other into the posterior parietal cortex, demonstrates that these two regions 'project to the same areas of limbic cortex (entorrhinal, parahippocampal, cingulate, retrosplenial and subicular) and to common targets in opercular and superior temporal cortical areas'. In addition, these areas are unified by their thalamic input from the medial pulvinar. The coincidence between this circuit described on the basis of labelling experiments in non-human primates and the brain regions implicated in the present study is remarkable. Furthermore, most of these regions have been shown to have abnormal cytoarchitecture in schizophrenia. Structural disorganization of hippocampal pyramidal cells (Kovelman and Scheibel, 1984) and a reduction of neuron content in entorrhinal cortex and hippocampal formation (Falkai and Bogerts, 1986; Falkai *et al.*, 1988) have been reported. Jakob and Beckmann (1986) report alterations to and loss of cells in the ventral insular and parahippocampal cortex. Abnormalities in the thalamus have also been reported. Laminar specific neuronal loss has been found in the frontal and cingulate cortex (Benes *et al.*, 1986).

One key difference between the two canonical profiles is the double dissociation in terms of prefrontal cortical involvement. The prefrontal neuroanatomical components shared by reality distortion and disorganization are medial prefrontal and anterior cingulate (with decrease in right frontal operculum). In contradistinction the left DLPFC is implicated in psychomotor poverty and, to a lesser extent, disorganization syndrome. The anterior cingulate has been shown to be involved with *attention* (Pardo *et al.*, 1990) and the left DLPFC with *intention* (Frith *et al.*, 1991). The DLPFC does not appear to contribute to attentional aspects of behaviour, indeed lesions of the DLPFC in non-human primates generally improve performance on sensory discrimination tasks (Irle, 1990). The DLPFC has been specifically associated with response selection in the absence of extrinsic information (Goldman-Rakic, 1987). This is a definition of intrinsically generated or willed behaviour. Reality distortion and disorganization syndrome, in the broadest sense, share monitoring deficits in monitoring extrapersonal events and attending selectively (Liddle and Morris, 1991). Conversely, psychomotor poverty and disorganization share intentional deficits in the generation of intentional representations and their translation into motor behaviour. These shared cognitive deficits may correspond to the different prefrontal regions the subsyndrome pairs have in common.

The left medial temporal lobe in schizophrenia

A recent review of neuropathology in schizophrenia (Roberts, 1991) begins, 'It is probable that all schizophrenics have abnormalities in the medial temporal lobe, which differ in degree but not in kind'. The present findings concur with this observation. Medial temporal lobe structures (hippocampal formation, amygdala and parahippocampal gyrus) have been the subject of intensive study. Apart from the cellular disorganization mentioned above (Kovelman and Scheibel, 1984; Falkai and Bogerts, 1986; Jakob and Beckmann, 1986; Falkai *et al.*, 1988) volumetric reductions have been a consistent finding (Bogerts *et al.*, 1985; Brown *et al.*, 1986; Jeste and Lohr, 1989; Bogerts *et al.*, 1991).

Roberts (1991) details the connections of the parahippocampal gyrus. These include Brodmann's areas 21/22 (middle and superior temporal gyrus), 9/46 (DLPFC), 24/32 (anterior cingulate), insula and claustrum, amygdala, ventral tegmentum, thalamus, central grey matter, caudate and putamen. These connected regions again include the neuroanatomical components implicated by the canonical analysis. There are multiple connections between the DLPFC and cingulate and the hippocampal formation. Of particular note is a prominent and reciprocal pathway between the principal sulcus (DLPFC) and the hippocampal formation by way of the caudomedial lobule which forms a bulge at the caudal extent of the hippocampal formation. This projection is important because it is more extensive than those to the entorrhinal and parahippocampal cortex and may be an important link that is highly developed in primates (Goldman-Rakic 1987). The highest correlations beween rCBF and psychopathology were seen medially at the caudal (posterior) extent of the hippocampal formation and may correspond to this structure. In a recent report of magnetic resonance imaging (MRI) of the left medial temporal lobe morphology in first episode schizophrenics, volumetric reductions (in males) were seen only in the left posterior limbic portion (Bogerts *et al.*, 1991) at a coronal level implicated in the present study.

The evidence for systematic anatomical changes in the left temporal lobe is compelling but does not provide a direct explanation for the present finding of *increased* regional activity with psychopathology. Atrophic changes or even loss of neural elements not detectable by MRI would, in a simple case, suggest a reduction in synaptic density and a progressive reduction in overall transynaptic activity and consequently a fall in neurophysiological correlates. The opposite was found. An obvious inference is that severe schizophrenia is associated with increased transynaptic activity (inhibitory or excitatory) mediating the transformation of neuronal firing patterns. In other words, a reduction in the efficiency of these transformations. The anatomical substrate of this positive physiological correlate may include atrophic changes, but the nature of the presumed dysplasia is such that it results in higher rCBF. A possible mechanism for the observed increases in medial temporal rCBF is disinhibition. Weinberger (1987), in an elaboration of the dopamine hypothesis, proposed an interaction between mesolimbic and mesocortical dopaminergic systems. He suggested that mesocortical (DLPFC) underactivity may result in subcortical disinhibition of dopaminergic activity. This model depends on a corticolimbic pathway which, despite initial findings (Pycock *et al.*, 1980) has yet to be identified (Christie *et al.*, 1986).

Cause or effect

Implicit in the above discussion is the idea that the common hyperactivity in the left parahippocampal region results from disinhibition and is a secondary phenomenon. This is tenable, given the massive and distributed connections of this area with other brain regions, any one of which may be dysfunctional. The alternative interpretation is that the left medial temporal lobe is the site of a primary abnormality causing remote dysfunction via interconnections. This interpretation is best accommodated by neurodevelopmental models. Abnormalities in cell migration during foetal development have been inferred from quantitative observations on the pre-alpha cell cytoarchitecture in the parahippocampal gyrus. Roberts (1991) reflects on the sequelae of these putative early anomalies, in schizophrenia the focus of the abnormality is the parahippocampal cortex and this stone in the developmental pond causes ripples which can spread to affect other brain areas.

A fixed dysplastic lesion does not explain 'many aspects of the disease, such as periodic relapses and recovery' (Bogerts *et al.*, 1991). However, the concept of a non-fixed dysplastic lesion as a consequence of abnormal parahippocampal development does allow for both primary and secondary parahippocampal effects as the neurodevelopmental 'ripples' are reflected by the cortex back onto the limbic system. Dysplastic mechanisms of this sort may persist into adulthood and beyond the onset of illness.

Neural plasticity in schizophrenia

Plastic changes in neurotransmission are now being characterized. Neural plasticity refers to the capacity of the nervous system to exhibit structural and functional adaptations to impinging stimuli. Long-term potentiation (LTP) is widely considered to be one of the best available models of plasticity that might underlie learning. The association between temporal lobe epilepsy and schizophrenic symptoms (e.g. Ounsted and Lindsay, 1981) again implicates over-activity in the medial temporal lobe and can be related to plastic mechanisms through a predisposition to kindling phenomena [*see* Cain (1989) for a comparison of LTP and kindling].

The particular relevance of neural plasticity to the present findings is at the level of neuroanatomical specificity of plastic potential. Mapping studies indicate that LTP and kindling phenomena are especially demonstrable in limbic regions. Hippocampal pathways show the strongest and most enduring LTP effects, but LTP is also seen throughout the limbic forebrain (Haracz, 1985). The regional specificity of plastic potential is confirmed by the finding of Benowitz *et al.* that the amygdaloid and hippocampal regions are among the few areas that express growth assóciated protein GAP 43 in the adult human brain, indicating that potential for plastic change, in this area, may extend into adulthood (Mesulam, 1990). Haracz (1985) has reviewed experimental evidence relating neural plasticity and schizophrenia.

In conclusion abnormalities in the left parahippocampal region appear to be a ubiquitous finding in schizophrenia. The present findings suggest overall severity of symptoms correlates highly with left parahippocampal rCBF. Further analysis suggests this abnormality is associated with dysfunction in remote association-paralimbic brain regions, particularly the prefrontal cortices. The prefrontal component of this distributed frontolimbic dysfunction is, unlike the left parahippocampal involvement, critically dependent on the behavioural subsyndrome.

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PRESENCE, DISTRIBUTION AND SPREAD OF PRODUCTIVE VARICELLA ZOSTER VIRUS INFECTION IN NERVOUS TISSUES

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SUMMARY

Nervous tissue lesions were retrospectively studied for detection of productive varicella zoster virus (VZV) infection in 33 autopsied cases, including 19 herpes zoster (HZ) (10 trigeminal, nine spinal) and 14 cases of nodular brainstem encephalitis without HZ. Immunocytochemistry for VZV antigens and *in situ* hybridization with a biotinylated VZV DNA probe were used on formol-fixed paraffin sections. Peripheral and central nervous system, skin and striated muscle were investigated in serial sections; available tissue blocks, however, varied between cases.

Varicella zoster virus production (both antigen and DNA) in nervous tissue was found in HZ cases but only of short survival after a rash of up to 7 wks (eight out of 12 patients). Varicella zoster virus was visualized in nerve cells, glial cells, Schwann cells and blood vessels. In the central nervous system (CNS), VZV was detected in trigeminal nuclei (one out of 10 brains) or disseminated nodular brainstem lesions (one out of 10 brains), in subependymal microvessels (one out of 10 brains) or vasculitic arteries (two out of 19 brains or spinal cords). In the peripheral nervous system (PNS), VZV (DNA and antigen) was found in neurons and satellite cells of sensory ganglia (four out of seven cases with sampling of ganglia), and in damaged nerve fibres including a muscle nerve in one case; myositis with VZV in affected muscle fibres was found in the latter case. In nodular brainstem encephalitis, one case contained VZV within nodular lesions.

We conclude that (i) VZV neural spread is suggested by detectable virus in ganglia, nerve fibres and CNS target nuclei; (ii) haematogenous spread of VZV is suggested by detection of virus in CNS microvessels and in disseminated brainstem encephalitis; (iii) VZV myositis may occur in zosteric myotomes; and (iv) VZV is a possible agent in nodular brainstem encephalitis.

INTRODUCTION

Varicella zoster virus (VZV) infects nearly 100% of the population in temperate parts of the world (Weller, 1983*a*,*b*). Fifty percent of individuals over 80 yrs develop herpes zoster (HZ) which is ninefold the frequency of this disease in the second decade (Gilden and Vafai, 1989). In view of the frequency and severity of life-threatening illness in the immuno-compromised host (Gilden and Vafai, 1989), any central nervous system (CNS) complication of infection with this 'not so benign' virus (Weller, 1983*a*,*b*) is important.

During chickenpox, VZV spreads into sensory ganglia via contiguous exteroreceptive nerves or by a haematogenous route (Gelb, 1990). Latent infection of sensory ganglia

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and reactivation are basic assumptions in the current model of zoster pathogenesis (Hogan and Krigman, 1973; Gelb, 1990).

Central nervous system involvement in HZ may present as encephalitis or encephalomyelitis (Appelbaum *et al.*, 1962; McCormick *et al.*, 1969; Hogan and Krigman, 1973; Ruppenthal, 1980; Horten *et al.*, 1981; Pilz and Budka, 1982; Ryder *et al.*, 1986). In addition, perivenous encephalomalacia (McCormick *et al.*, 1969), leukoencephalopathy (McCormick *et al.*, 1969; Horten *et al.*, 1981; Ryder *et al.*, 1986) with (Ryder *et al.*, 1986) or without brainstem involvement (McCormick *et al.*, 1969; Horten *et al.*, 1981) were observed. Sometimes similarities to progressive multifocal leukoencephalopathy (Horten *et al.*, 1981) and granulomatous angiitis of the nervous system (Rosenblum and Hadfield, 1972; Linnemann and Alvira, 1980; Ruppenthal, 1980; Eidelberg *et al.*, 1986) have been noted. Varicella zoster virus aetiology of lesions was concluded from Cowdry type A inclusions in glial cells (McCormick *et al.*, 1969; Hogan and Krigman, 1973; Ruppenthal, 1980; Horten *et al.*, 1981; Pilz and Budka, 1982; Ryder *et al.*, 1986), in ependyma (Pilz and Budka, 1982), in neurons (Horten *et al.*, 1981; Pilz and Budka, 1982; Ryder *et al.*, 1986), in granulomatous angiitis (Linnemann and Alvira, 1980; Ruppenthal, 1980; Eidelberg *et al.*, 1986) and in arachnoid cells (Ruppenthal, 1980; Pilz and Budka, 1982). More specific data included detection of herpes virions by electron microscopy (McCormick *et al.*, 1969; Hogan and Krigman, 1973; Horten *et al.*, 1981; Ryder *et al.*, 1986), of VZV antigens by immunocytochemistry and of genome by Southern blot hybridization (Ryder *et al.*, 1986) and by polymerase chain reaction (Mahalingam *et al.*, 1990).

This retrospective study on human nervous tissue obtained at autopsy was carried out to determine the presence, distribution and spread of VZV in tissues of HZ patients and in nodular brainstem encephalitis by histology, immunocytochemistry and *in situ* hybridization.

MATERIALS AND METHODS

Thirty-three human autopsy cases including 19 with HZ and 14 with nodular brainstem encephalitis of as yet undetermined aetiology were studied. Most of the patients died from causes unrelated to VZV (Table 1).

Sixteen HZ cases were reported in a previous histopathologic study (Pilz and Budka, 1982) in which immunocytochemistry and *in situ* hybridization for VZV were not performed All cases examined in this study had complete neuropathological investigation of numerous tissue blocks. Survival of the HZ cases after the rash ranged between 1 wk and 5 yrs. Nervous tissue available for investigation of VZV production in HZ is detailed in Table 1. Myotomal muscle (one case) and skin affected by the rash (two cases) were also studied. Nodular brainstem encephalitis cases were previously studied by histology, immunocytochemistry and *in situ* hybridization for detection of cytomegalovirus (CMV) (Schmidbauer *et al.*, 1989*a*) and herpes simplex virus (HSV) (Schmidbauer *et al.*, 1989*b*). From the original nodular brainstem encephalitis series, 14 cases without presence of CMV and HSV were investigated for VZV. Cases with CMV (five cases) or HSV (five other cases) from the same series were used as specificity controls Seven patients with nodular brainstem encephalitis had AIDS.

After selection of blocks with histopathological lesions, serial sections of each block were examined by routine histological stains, immunocytochemistry and *in situ* hybridization for VZV. Additional immunocytochemistry for a variety of cellular markers was performed in selected blocks for determination of the type of infected cells. Four to six blocks were studied in each trigeminal HZ case, two to six blocks were studied in each spinal HZ case and two blocks were studied in each nodular brainstem encephalitis case

Specificity controls included one case each of connatal cytomegalic inclusion body disease and acute necrotizing HSV encephalitis, and five cases each of nodular encephalitis with demonstrable CMV or HSV, respectively. Ten negative tissue controls (age range 6 mths to 76 yrs) included various degenerative, vascular, inflammatory and metabolic diseases of the brain. Varicella zoster virus was not detected in any controls by immunocytochemistry or *in situ* hybridization.

Immunocytochemistry for VZV

Four different mouse monoclonal antibodies (ascitic fluid) were produced in the University of Tokyo, Japan. They were used at dilutions of 1:500 and 1:2000. Two (cl. 8-1 and cl. 8-2) recognized different epitopes of glycoprotein 3 (gp 3) of VZV (Okuno *et al* , 1983), two (cl. 17-1 and cl 17-2) recognized different epitopes of nucleoprotein (np). Sections were stained by the labelled avidin-biotin peroxidase complex technique after enzymatic predigestion with protease; diaminobenzidine was used as chromogen. Nuclear counterstaining used haemalum

In situ hybridization for VZV

A biotinylated DNA probe (VZV/H-N3 DNA/*Eco*RI D fragment) (Hondo *et al.*, 1987) was used in a concentration of 10 ng/μl. Paraffin-embedded sections of 3–5 μm thickness were hybridized as described previously (Schmidbauer *et al.*, 1989*b*). For visualization, the following amplification was used: rabbit antibiotin served as bridging antiserum (dilution 1:1000) in 0.5% blocking reagent (Boehringer) in 100 mM Tris and 150 mM NaCl at H 7.5+3% human serum+10% swine serum. Subsequently, a standard three step peroxidase-antiperoxidase (PAP) method for detection of rabbit immunoglobulins was performed. Slides were washed with water and counterstained with haemalum.

RESULTS

Herpes zoster

The age of patients with diagnoses other than HZ, causes of death, localization of the rash, survival after the zoster skin lesion, tissues examined for VZV production and the results of immunocytochemistry and *in situ* hybridization for VZV are summarized in Table 1. In all cases with detectable VZV products, both DNA and antigens were found.

Skin. Two cases of florid necrotizing HZ were investigated. Cytopathic changes in the epidermis were acantholysis, syncytial formation (Fig. 1A,B,C) and focal necrosis which extended into adjacent connective tissue. Marked lymphocytic infiltrates surrounded intact or necrotic blood-vessels. Cowdry type A intranuclear inclusions were found in epidermal cells (Fig. 1A,B,C), in fibroblasts and in cells of blood-vessel walls and of sweat glands. These nuclear inclusions contained VZV DNA (Fig. 1C) and VZV np (Fig. 1A). The cytoplasm contained large amounts of VZV gp 3 (Fig. 1B) but small amounts of VZV np and VZV DNA. Some epidermal cells without inclusions also contained VZV products. Some cutaneous nerves harboured VZV (Fig. 1D). Even remote from herpetic lesions, VZV production was documented in cells of blood-vessel walls (Fig. 2) in the subcutis.

Sensory ganglia, nerve roots and peripheral nerve. All segmental ganglia of seven HZ patients showed histopathologic changes which ranged from degeneration of ganglion cells with lympho-plasmocellular infiltration and sclerosis to florid necrosis with Cowdry type A inclusions in ganglion cells and in satellite cells (Fig. 3B). Inclusions were also found in satellite cells remote from ganglion cell necrosis. In one case, lymphocytic infiltrates extended through the perineurial and epineurial sheaths of the dorsal spinal root to the leptomeningial space. Dorsal root nerve fibres showed degeneration. Varicella zoster virus products (four out of seven cases) were found in Cowdry type A inclusions

TABLE I

TRIGEMINAL ZOSTER

Clinical data						*Neuropathology and presence of VZV*						
Case no.	*Age*	*Diagnosis other than HZ*	*Cause of death*	*Loc*	*Onset*	*Periph. n*	*Ggl G*	*Ncl. mesenc*	*Ncl princ*	*Ncl. spin.*	*Dissem E*	*Other lesions*
1	75	Tuberculosis	Pneumonia	V1	n.d.	n.i.	n.i.	0	n.i.	*	*	Vasculitis, meningitis
2	75	Cerebral sclerosis	Pneumonia	V2/3	2 wks	n.i.	n.i.	0	n.i.	*	*	0
3	79	Heart dilatation	Heart failure	V1 → gen	3 wks	n.i.	n.i.	0	n.i.	*	*	0
4	71	Leukaemia	Generalized zoster	V1 → gen	17 d	n.i.	n.i.	0	n.i.	*	*+	Microvasculitis*+
5	84	Liver disease	Pulmonary embolism	V1	1 wk	n.i.	n.i.	n.i.	*	*	*	0
6	72	Heart dilatation	Pulmonary embolism	V1	1 wk	n.i.	n.i.	0	*+	*+	*	Meningoencephalitis
7	79	n d	n d	V1	5 wks	*+	*+	n.i.	*	*	*	Myositis*+
8	85	Myocardial infarction	Heart failure	V1	4 wks	n.i.	*+	0	*	*	*	0
9	81	Marasmus senilis	Pulmonary embolism	V1	n d	n.i.	n.i.	0	0	0	*	0
10	80	CLL	Generalized zoster	V1 → gen	n.d	n.i.	n.i.	0	*	*	*	0

SPINAL ZOSTER

Clinical data						*Neuropathology and presence of VZV*					
Case no.	*Age*	*Diagnosis other than HZ*	*Cause of death*	*Loc*	*Onset*	*Periph n*	*Sp G*	*Entry zone*	*Ncl. G/ Ncl B*	*Dissem M*	*Other lesions*
11	63	Pneumonia	Heart failure	Th 8	8 wks	n.i.	n.i.	n.i.	*	n.i.	Encephalitis
12	37	M.Hodgkin	Pneumonia	Cc → gen.	4 wks	n.i.	n.i.	n.i.	*	n.i.	Vasculitis*+, meningitis
13	78	n d	n.d	L5	7 wks	n.i.	*+	*	*	0	Vasculitis, meningitis
14	90	Kachexia	n d.	L1	3 wks	n.i.	*	*	*	0	Meningitis
15	78	Myocardial infarction	Heart failure	Th 4–6	n d	n.i.	*	*	*	*	0
16	67	St post rhizotomy	n d	L1	5 yrs	n.i.	n.i.	0	0	n.i.	0
17	65	Mamma-ca.	Pulmonary embolism	L2	1 yr	*	*	*	0	0	0
18	78	Hypernephroma	n d.	Th 10	8 d	*+	n.i.	n.i.	n.i.	n.i.	Meningitis
19	90	n.d	Generalized zoster	S4 → gen	9 d	*+	*+	n.i.	n.i.	n.i.	Men-ventr*, vasculitis*

Loc. = localization of skin rash; Onset = onset of skin rash prior to death, Periph. n = peripheral nerve; Ggl. G = Gasserian ganglion; Sp. G = spinal ganglion, Ncl mesenc. = nucleus mesencephalicus nervi trigemini, Ncl. princ. = nucleus principalis nervi trigemini; Ncl. spin. = nucleus spinalis nervi trigemini; Ncl G = nucleus Goll (gracilis); Ncl B = nucleus Burdach (cuneatus); Dissem. E = disseminated brainstem encephalitis; Dissem. M = disseminated myelitis; Men -ventr. = meningoventriculitis; n.d = no data available; gen. = secondarily generalized, n.i. = not investigated; 0 = no lesion, no labelling, * = histopathological lesion, no labelling; *+ = histopathological lesion with labelling (DNA+AG); CLL = chronic lymphatic leukaemia; Cc = coccygeal

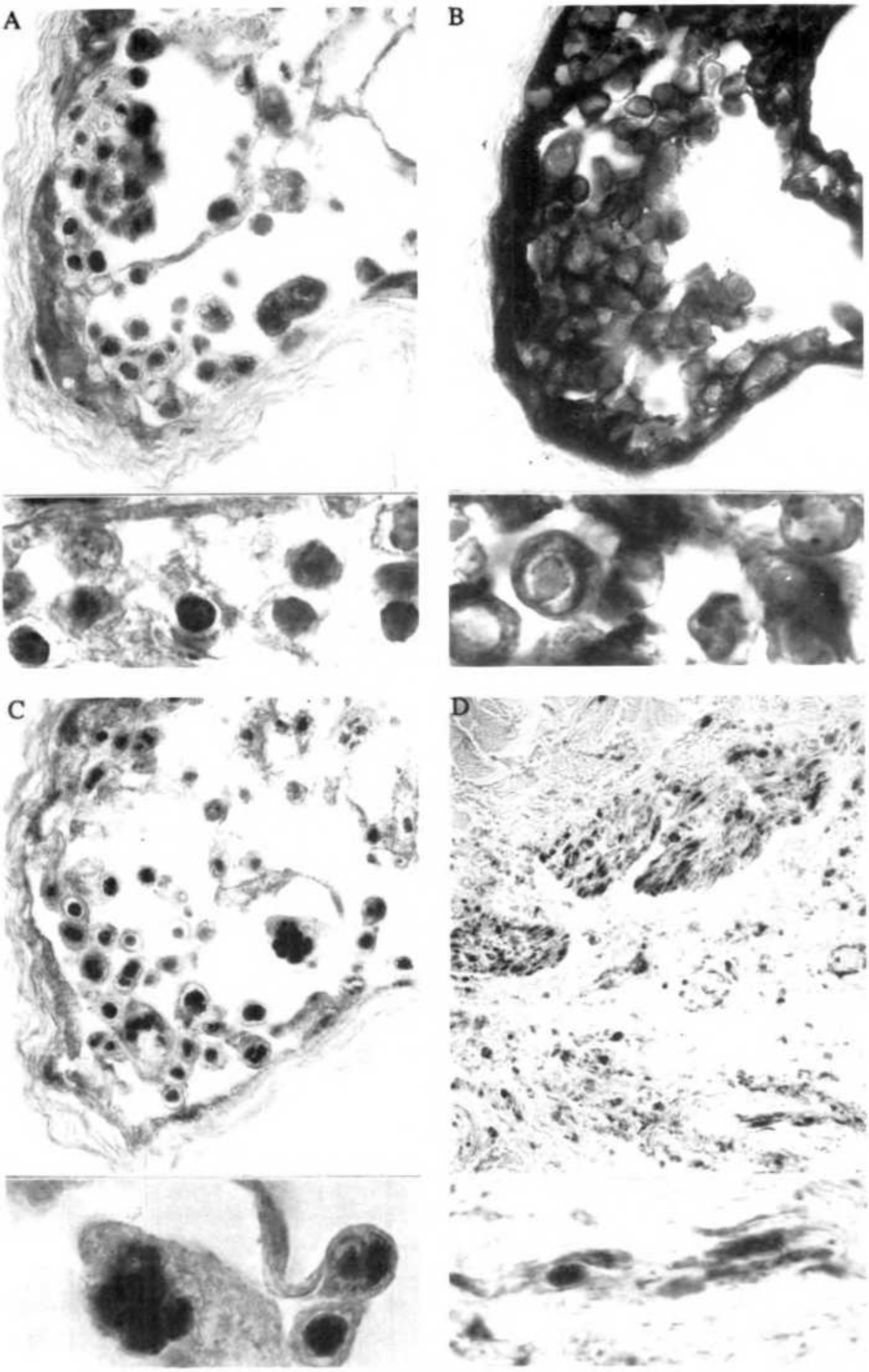

FIG. 1. Varicella zoster virus production in the skin (*Case 18*). A–C, epidermis with acantholysis and syncytia (inset in C) formation. Cowdry type A intranuclear inclusions in epidermal cells contained VZV np (A) and DNA (C) but little gp 3 (B). Cytoplasms of epidermal cells contain large amounts of VZV gp 3 (B) and variable amounts of np (A) antigens. Immunocytochemistry for VZV np (A, cl. 17-1) and gp 3 (cl. 8-1) antigens. *In situ* hybridization with biotinylated cDNA probe (C). Haemalum counterstain. Magnifications ×320, insets ×800. D, dermal nerve beneath bullous epidermal lesions contains VZV np antigens in elongated structures, probably representing Schwann cells. Fibroblast nuclei within connective tissue of the corium are also labelled. Immunocytochemistry with cl. 17-2. Magnification ×144, inset (from same field) ×800.

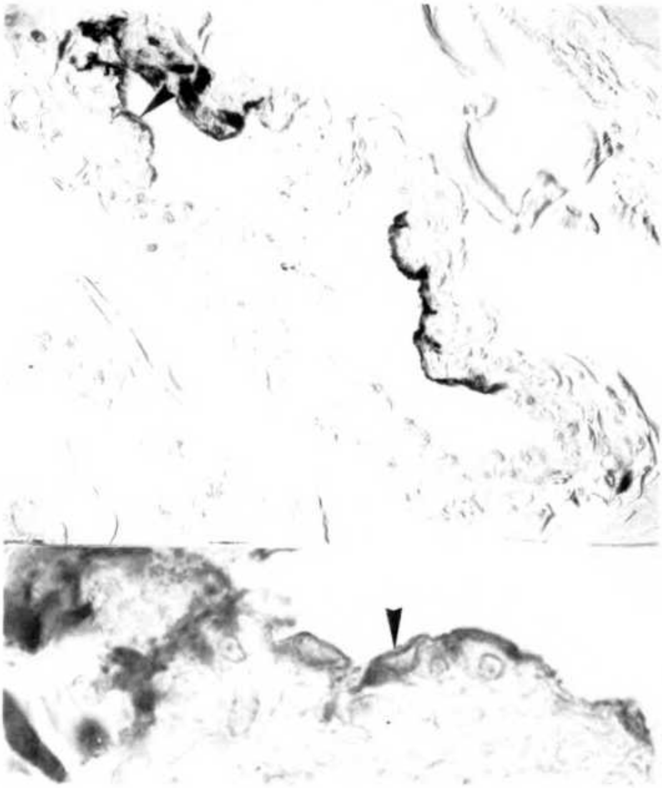

FIG. 2. Varicella zoster virus production in subcutaneous vasculature (*Case 19*). Subcutaneous vein, remote from the epidermal herpetic ulcer, contained gp 3 antigen in cytoplasms of vessel wall cells; nuclei are spared. Arrowheads mark the same cell in both magnifications. Immunocytochemistry with cl. 8-1. Haemalum counterstain, Nomarski optics. Magnification ×250, inset (from same field) ×1000.

in ganglial cells and in satellite cells (Fig. 3B), in damaged nerve roots, in vasa nervorum and in connective tissue (Fig. 3A). In one case, a distal peripheral nerve contained large amounts of VZV in endo-, peri- and epineurium (Fig. 3C), and in Schwann cells. Varicella zoster virus products were also found along necrotic nerve fibres. Virus was not detected in non-necrotic ganglionic tissue, either normal or with inflammation or secondary degeneration.

Sensory projection areas of CNS. In trigeminal HZ, histological tissue damage in the brainstem included: (i) focal encephalitis of the spinal nucleus and of the nucleus sensorius principalis nervi trigemini, respectively (nine out of 10 cases); (ii) mild, disseminated bulbo-ponto-mesencephalic encephalitis (all 10 cases examined) (Table 1).

In both focal trigeminal and disseminated encephalitis, glial nodules and degeneration of myelin with lymphocytic infiltration were found. Inclusion bodies were seen in four cases. Focal trigeminal encephalitis featured small necroses in one case. Varicella zoster virus products were detected in glial cells, with or without inclusions (in one case) (Fig. 4). The mesencephalic trigeminal nucleus did not contain detectable VZV nor did it show histopathological changes.

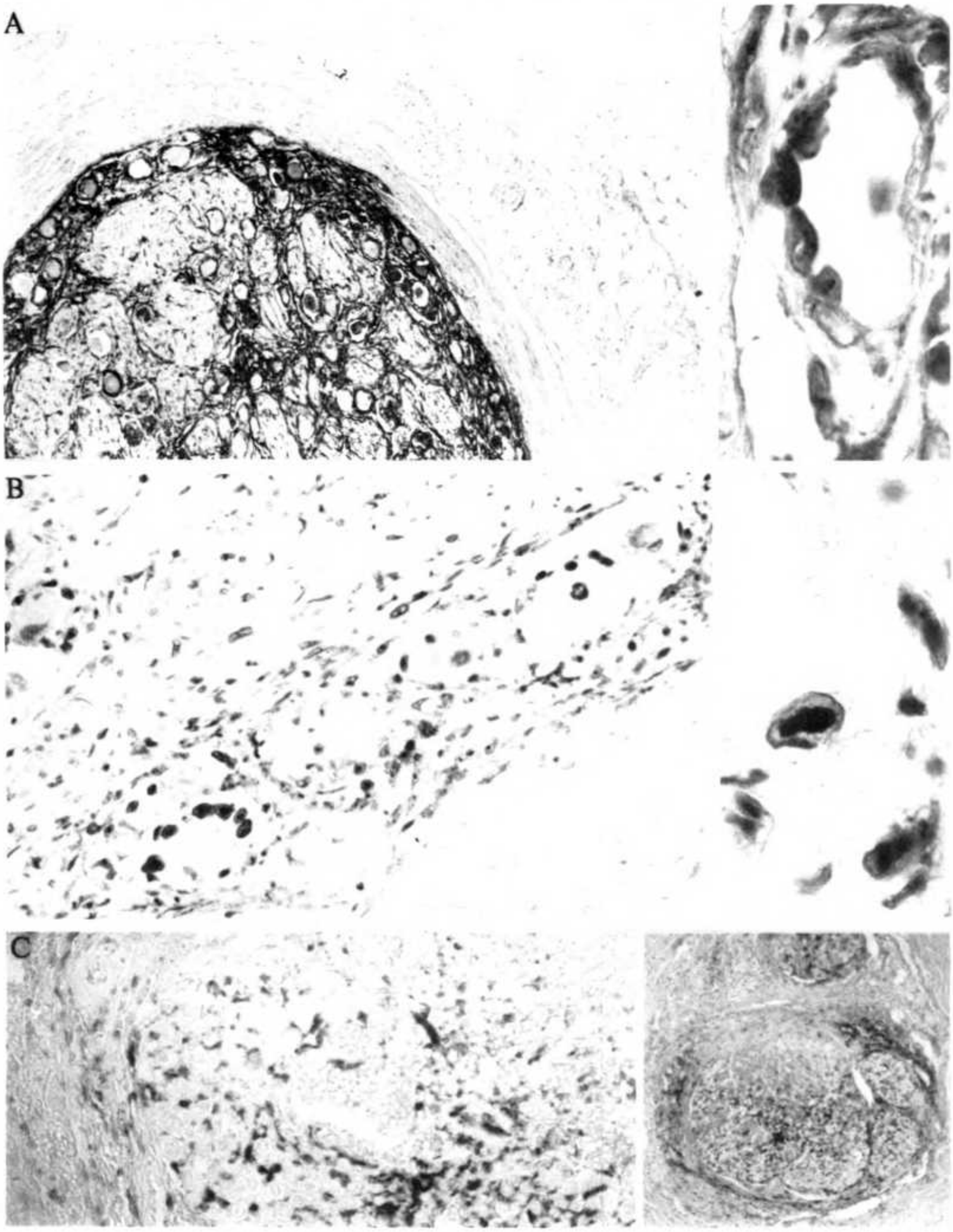

FIG. 3. Varicella zoster virus production in the peripheral nervous system (*Case 19*). A, necrotizing ganglionitis with abundant VZV antigens, involving also a vessel (inset). Immunocytochemistry with anti-gp 3 antibody (cl. 8-1), haemalum counterstain. Magnification ×63, inset ×840. B, nuclei of satellite cell, intranuclear inclusions of ganglionic cells, and fibroblastic nuclei in ganglionic endoneurium contain VZV antigens. Immunocytochemistry with anti-np antibody (cl. 17-1), haemalum counterstain. Magnification ×151, inset ×840. C, spinal nerve with focal necrosis. Endo-, peri- and epineurial presence of VZV antigens. Lymphocytic inflammatory infiltrates extend to the epineural surrounding tissues. Immunocytochemistry with anti-gp 3 antibody (cl. 8-1), haemalum counterstain. Magnification ×210, inset (from same field) ×25.

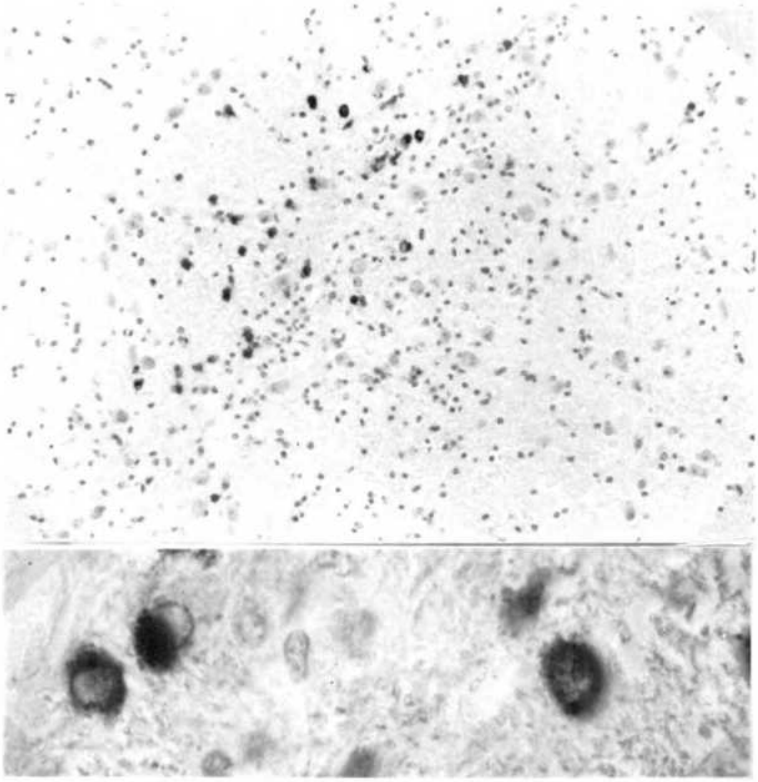

FIG. 4. Varicella zoster virus production in the brainstem (*Case 6*). Nodular pontine lesion with inflammatory infiltration in a patient with trigeminal zoster. Many cells are labelled for VZV antigens. Immunocytochemistry with anti-gp 3 antibody (cl. 8-2). Haemalum counterstain. Original magnification ×75; inset (from same field) ×1000.

In spinal HZ, histopathological lesions were found in primary sensory projection areas in the dorsal horn (four out of five cases with this sampling), and in the gracile and cuneate nuclei (five out of seven cases). They were indistinguishable from those of focal and disseminated brainstem encephalitis in trigeminal HZ. However, inclusion bodies and VZV were not detected in the spinal cord.

Some motor neurons in trigeminal HZ, and some anterior horn neurons in spinal HZ segments showed central chromatolysis; VZV was not detectable in these cells.

Ependyma and arachnoidal cells. Cowdry type A inclusion bodies were found in ependyma and arachnoidal cells of one case with secondarily generalized sacral HZ (Case 19); unfortunately, sections of ependyma and leptomeninges were not available for immunocytochemistry and *in situ* hybridization in this case.

Subependymal areas. In one case of disseminated bulbo-ponto-mesencephalic encephalitis after secondarily generalized trigeminal zoster (Case 4, Table 1), VZV was detected in the walls of small blood-vessels around the upper aqueduct and the adjacent third ventricle (Fig. 5A) and in perivascular glial cells. The type of infected cells in vascular walls could not be identified beyond doubt.

Meningitis. In areas with severe lympho-monocytic infiltration of the leptomeninges

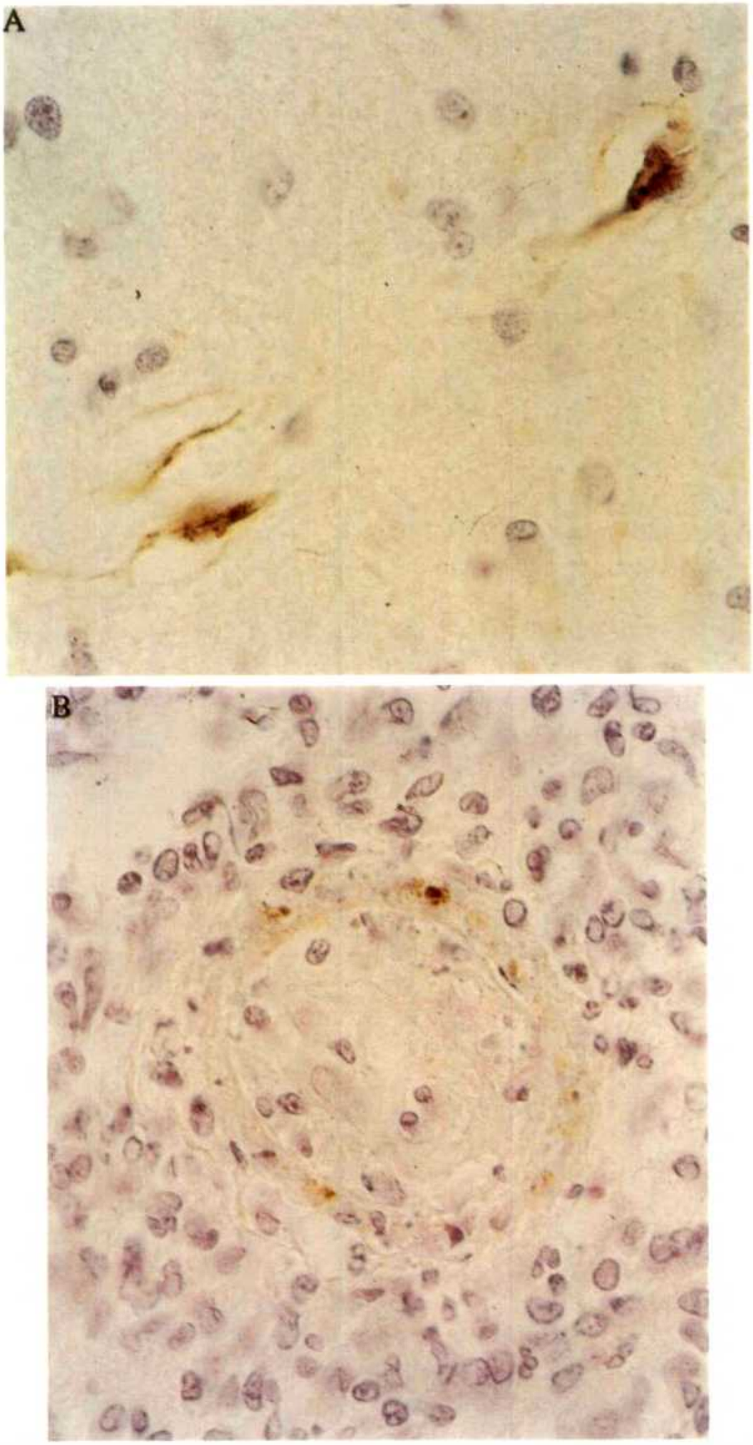

FIG. 5. Varicella zoster virus production in cerebral vessels (*Case 4*). A, subependymal microvasculature along the caudal third ventricle contained VZV antigen in the vessel wall cells. Immunocytochemistry with anti-gp 3 antibody (cl. 8-1), haemalum counterstain. Magnification ×1000. B, necrotizing vasculitis in a subarachnoid blood-vessel with thrombotic occlusion. Varicella zoster virus antigen was detected in the necrotic tunica media. Periadventitial lymphocytic infiltration was present. Immunocytochemistry with anti-np antibody (cl. 17-1), haemalum counterstain. Magnification ×1000.

(seven cases), inflammation encroached on neighbouring superficial brain tissue or cranial nerves, with local presence of VZV in glial cells and Schwann cells in one case.

Vasculitis. Four (one trigeminal, three spinal) of 19 HZ cases had vasculitis. In the trigeminal HZ case (Case 1, Table 1), lymphocytic infiltrates were found within the walls of medium sized and small veins without detectable VZV; meningitis was present in this case. In a case of coccygeal zoster with secondarily generalized rash (Case 12, Table 1), multiple fibrinoid necroses were found in the walls of arteries in the brainstem and cerebellum; VZV was present within the walls of such vessels in this case (Fig. 5B). Necroses extended into the intraparenchymal and endoneural segments of subarachnoid arteries. Thrombangiitic occlusions in these vessels were common, and disseminated infarction of the brainstem and cerebellum was extensive and included optic chiasm, olfactory tracts and bulbs, and oculomotor nerve roots. A second case of spinal HZ (Case 19) had necrotizing vasculitis with detection of VZV restricted to ganglionitis and distal peripheral nerve. In another spinal HZ case (Case 13) necrotizing vasculitis with local infarction but without detectable VZV was seen in the close vicinity of infected ganglia and in the entry zone of the dorsal root. The nature of individual infected cells within vessel walls could not be established unequivocally even when using immunocytochemistry for endothelial and smooth muscle markers on adjacent serial sections.

Muscle. Scattered areas in masseter muscle (Case 7) showed severe perivascular and endomysial lymphocytic infiltration. Some muscle fibres were atrophic or showed proliferation of sarcolemmal nuclei. Varicella zoster virus was detected in inclusions of a few muscle fibre nuclei (Fig. 6). Intramuscular nerves showed focal degeneration of myelinated fibres and lymphocytic infiltrates of epineurial and perineurial connective tissue. Varicella zoster virus was detected in the perineurium of intramuscular nerves.

Nodular brainstem encephalitis without herpes zoster

Among the 14 nodular brainstem encephalitis cases, VZV antigens and DNA were detected within nodular lesions of one brain. This interesting case is reported in more detail. Five days before death, the 20-yr-old male experienced headache and fever and collapsed during a military exercise. Speech disturbance and epileptic seizures followed. A corneal lesion, cyanosis due to pneumonia, and diarrhoea developed. The patient became comatose. Temperature was 40.5 °C; leukocytosis of 20.000/mm^3 was present. Cerebrospinal fluid (CSF) was normal. He finally developed bleeding diathesis, signs and symptoms of hypodynamic shock and rhabdomyolysis. Renal and hepatic failure occurred despite plasma exchange, haemodialysis and blood transfusion. No rash was visible. Stool, blood, urine and CSF cultures for bacteria were all negative. Serological and CSF investigations for neurotropic viruses, including complement fixation tests for VZV, were negative. Autopsy disclosed pneumonia, severe enteritis and myocardial dilatation. The brain was swollen and there was venous congestion. Cerebral and cerebellar hemispheres were normal. In the brainstem, some glial cells within encephalitic nodules were labelled for VZV antigen (Fig. 7) and DNA. Cells with definite intranuclear inclusions were not present. Histological investigations were not carried out on other organs.

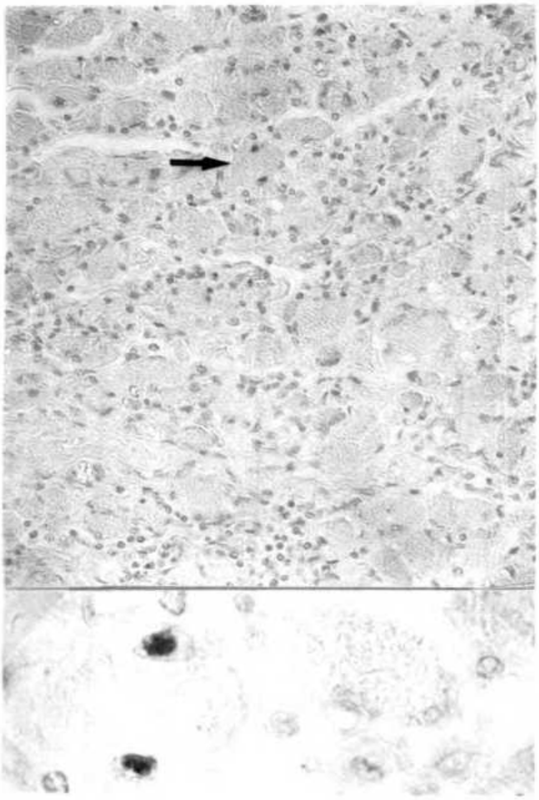

FIG. 6. Varicella zoster virus myositis (*Case 7*). Masseter muscle with prominent inflammatory infiltration. Varicella zoster virus antigens are detected in inclusions in two nuclei of one muscle fibre (arrow and inset). Immunocytochemistry with anti-np antibody (cl. 17-2), haemalum counterstain. Original magnification ×160, inset ×890.

DISCUSSION

This is a detailed retrospective study of a series of HZ patients correlating histopathology with detection of VZV in nervous tissue by immunocytochemistry and *in situ* hybridization. Unfortunately, tissue sampling was not uniform, due to restricted availability. Thus conclusions concerning the general mechanisms of VZV related neuropathology are limited. No correlation was shown between the frequency of histopathological lesions and the frequency of detectable VZV production in HZ in the nervous system. Possible explanations include:

(i) Intervals between florid HZ and death ranged up to 5 yrs, so that absence of VZV, detectable by immunocytochemistry and *in situ* hybridization, may be attributed to burning out in long-standing cases. When only the short survivals after the rash (up to 7 wks) were considered (12 cases), two-thirds (eight cases) had VZV production detectable in one (n = 4), two (n = 2) or three (n = 2) localizations within the nervous system.

(ii) Small lesions in the CNS of HZ cases may have escaped detection. Indeed, only the margins of lesions were seen in those slides which were examined by immunocytochemistry and *in situ* hybridization in 50% of cranial and 44.4% of spinal zoster. If we consider only immunocytochemistry and *in situ* hybridization slides with definite histopathology in cases of short survival (up to 7 wks), then VZV production was demonstrated in nervous tissue of 80% of cases.

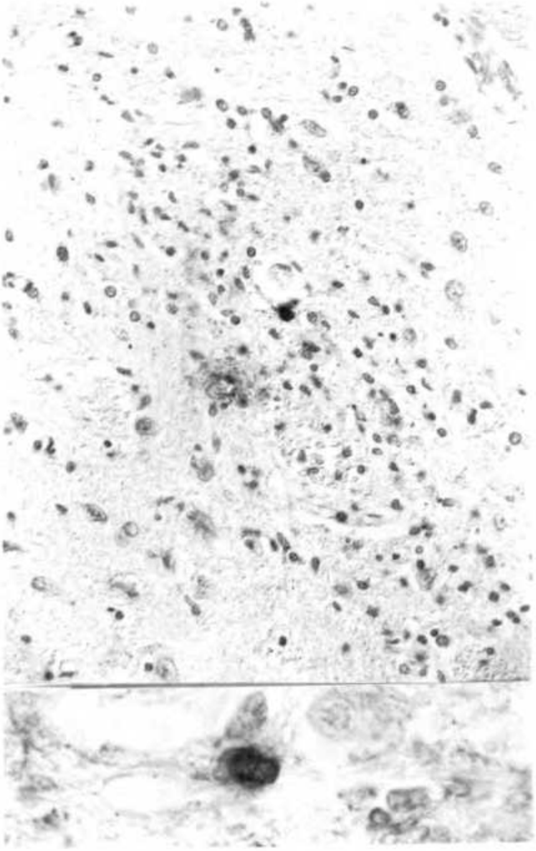

FIG. 7. Varicella zoster virus production in nodular brainstem encephalitis. Inflammatory lesion in the medulla oblongata in nodular brainstem encephalitis. One cell was labelled for VZV antigen; immunocytochemistry with anti-np antibody (cl. 8-2), haemalum counterstain. Magnification ×75, inset ×1000.

(iii) Small amounts of VZV may have remained undetected due to lack of sensitivity of our immunocytochemistry and *in situ* hybridization techniques for human autopsy material, as discussed in previous studies for other herpes viruses (Aksamit *et al.*, 1985; Unger *et al.*, 1986; Schmidbauer *et al.*, 1988, 1989*a*). The more sensitive polymerase chain reaction technique indicated latent presence of VZV DNA (Mahalingam *et al.*, 1990) but failed to distinguish virus production.

Several characteristic distribution patterns of VZV pathology in individual HZ patients suggest a number of different ways of virus spread: in necrotizing zoster ganglionitis, we saw focal inflammation of ganglion cells extending to the perineurium and epineurium and leading to local meningitis. These findings may explain the high incidence of CSF pathology in HZ (Appelbaum *et al.*, 1962). Spread of inflammation via the *meningeal ramus* of the zoster ganglion is an alternative initial step to meningitis (Ruppenthal, 1980; Pilz and Budka, 1982).

The theory that VZV spreads along exteroceptive nerve fibres to the skin is supported here by the detection of virus in peripheral nerve of segments affected by zoster in two cases of our series. Infection of local cutaneous blood-vessels, as documented here, may lead to viraemia with consecutive haematogenous dissemination. Our observation of the presence of VZV in the circumventricular microvasculature, remote from local sensory projection areas, in generalized zoster without infection of meninges or ependyma, might be a sequel of such virus spread. Entry of virus into the CNS may be relatively easy via blood-vessels of circumventricular organs which do not have the tight junctions of the normal blood brain barrier (Davson *et al.*, 1987; Tyler and Fields, 1989).

Myositis in herpes zoster was described by Norris *et al.* (1969), but viral particles were not herpes-like. In a modern textbook on neuromuscular diseases (Swash and Schwartz, 1988), VZV is not mentioned as a possible agent in myositis. However, in this study, VZV was demonstrated in myositic masseter muscle of trigeminal HZ. Since skeletal muscle is not a target of infection in chickenpox (Gelb, 1990), local reactivation of VZV seems unlikely. Neural VZV spread to muscle, or haematogenous spread during 'silent viraemia', is a possibility.

We observed that myositis was restricted to the myotomes of the zoster segment, as did Norris *et al.* (1969) in their case report. This is evidence against haematogenous spread. Infection of proprioceptive ganglion cells may result in ganglio-muscular disease, in the same way as the postulated ganglio-cutaneous exteroceptive route.

Focal necrotizing vasculitis in the close vicinity of HZ necrotizing ganglionitis may indicate local spread of infection (Kuroiwa and Furukawa, 1981; MacKenzie *et al.*, 1981). However, we observed also disseminated necrotizing vasculitis and thrombangiitis with VZV scattered in all parts of the vessel walls at sites which were remote from the zoster focus, after secondary generalization of rash. This indicates haematogenous generalization of infection. Defects in cell mediated immunity in particular patients are probably instrumental in causing haematogenous dissemination (Gelb, 1990).

Postherpetic neuralgia is a clinical complication of HZ with a high frequency in the elderly and often represents a difficult management problem. In previous morphologic studies on possible correlates of post-herpetic pain, histology showed lymphocytic infiltration in the posterior spinal root, the posterior horn and the leptomeninges (Denny-Brown *et al.*, 1944), sclerosis of affected ganglia (Head and Campbell, 1900; Denny-Brown *et al.*, 1944; Watson *et al.*, 1988) and nerve fibre degeneration in spinal dorsal roots (Head and Campbell, 1900; Denny-Brown *et al.*, 1944; Zacks *et al.*, 1964; Watson *et al.*, 1988). All these light microscopic findings were present in the long survivors of our study. However, continuing production of VZV could not be demonstrated. A central mechanism, based on damage of the spinal trigeminal nucleus, has been assumed in postherpetic trigeminal neuralgia: spinal trigeminal involvement has been confirmed experimentally (King and Meagher, 1955; Crue *et al.*, 1956, 1964; King *et al.*, 1956). In keeping with these observations, tissue damage was found in the spinal trigeminal nucleus in all but one case of trigeminal zoster.

The presence of VZV production in nervous tissue of two-thirds of acute HZ patients (this study) is in contrast with similar recent work which failed to demonstrate VZV in nervous tissue by immunocytochemistry and *in situ* hybridization (Kennedy *et al.*, 1990). This discrepancy may reflect differences in the sensitivity of methods used and in the extensiveness of tissue sampling. Kennedy *et al.* (1990) studied representative blocks of brain and/or spinal cord, as well as a limited number of spinal ganglia, but no further details of these negative cases were given. Our study supports a direct role for the virus in the development of neuropathological lesions. However, other pathogenic mechanisms, such as immunopathogenesis supported by increased class II antigen expression (Kennedy *et al.*, 1990) may replace, accompany or follow direct action of the virus on nervous tissue.

The aetiology of nodular brainstem encephalitis has been obscure. Recently, we have demonstrated herpes simplex virus type I (HSV 1) DNA in 47% of brains with nodular brainstem encephalitis (Schmidbauer *et al.*, 1989*a*). Moreover, we found cytomegalovirus DNA within lesions of one-third of nodular brainstem encephalitis cases in AIDS (Schmidbauer *et al.*, 1989*b*). The present observation of VZV products within nodular brainstem encephalitis lesions of one previously healthy man adds VZV to the possible aetiological spectrum of nodular brainstem encephalitis. We conclude that:

(i) An exclusive route of VZV infection of nervous tissues is not demonstrable in this series. Suggested patterns of CNS involvement include: (*a*) infection of CSF spaces by direct extension from infected ganglia or via the meningeal *ramus*, with secondary meningoencephalitis and/or disseminated angiitis; (*b*) focal brainstem encephalitis or myelitis in primary sensory target nuclei by neural spread of infection; (*c*) disseminated brainstem encephalitis due to spread of focal infection originating as described in (*b*), or following haematogenous dissemination; (*d*) infection of subependymal microvasculature in viraemia.

(ii) Varicella zoster virus is produced in neurons, glial cells, Schwann cells, fibroblasts, striated muscle, epidermal cells, sweat glands and blood-vessels (mostly in cases with generalized rash); thus VZV is able, in principle, to infect permissively a broad range of cells and tissues. However, vulnerability of certain cell populations may depend upon mode of virus propagation and immune competency.

(iii) Myositis in the myotomes of affected zosdter segments can be caused by VZV.

(iv) Varicella zoster virus is a possible agent in nodular brainstem encephalitis.

(v) In our hands, *in situ* hybridization and immunocytochemistry are methods of comparable sensitivity to detect productive VZV infection in paraffin sections, even when Cowdry type A inclusions are not found.

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THE CLINICAL AND EPIDEMIOLOGICAL PROFILE OF LYME NEUROBORRELIOSIS IN DENMARK 1985–1990

A PROSPECTIVE STUDY OF 187 PATIENTS WITH *BORRELIA BURGDORFERI* SPECIFIC INTRATHECAL ANTIBODY PRODUCTION

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SUMMARY

This prospective study reports the clinical and epidemiological features of 187 consecutive patients with neuroborreliosis recognized in Denmark over the 6-yr period, 1985–1990. Only patients with intrathecal *Borrelia burgdorferi* specific antibody synthesis were included. In 1990 regional incidences varied between 5.7 and 24.1 per million. Ninety-four percent of the patients had early (second stage) neuroborreliosis. The most common manifestation was a painful lymphocytic meningoradiculitis (Bannwarth's syndrome) either with paresis (61%) or as a radicular pain syndrome only (25%). Central nervous system (CNS) involvement in early neuroborreliosis was rare; 4% had signs of myelitis and only one patient had acute encephalitis. Children showed a different course of the disease. Six percent of the patients suffered a chronic course with a disease duration between 6 mths and 6 yrs either as chronic lymphocytic meningitis (1.6%) or as third stage chronic encephalomyelitis (4.3%). Meningeal signs were rare despite pronounced inflammatory cerebrospinal fluid (CSF) changes (median cell count 160/μl; median protein concentration 1.13 g/l). High dose i.v. penicillin G was administered to 91% of the patients. Based on the clinical outcome and normalization of CSF no treatment failures were recognized. The final morbidity after a median follow-up of 33 mths was low; disabling sequelae were reported in nine patients, mainly those with previous CNS involvement. We conclude that neuroborreliosis is a common and characteristic neurological disorder. The diagnosis should be based on the demonstration of inflammatory CSF changes and *B. burgdorferi* specific intrathecal antibody production.

INTRODUCTION

Neuroborreliosis is a frequent manifestation of the tick-borne spirochetosis Lyme borreliosis and is being increasingly recognized in Europe and the USA. Neuroborreliosis may develop either as a second or third stage manifestation of this multisystemic infection. Although the aetiology was unknown until recently, several of the neurological manifestations have been described in Europe throughout the last 70 yrs (Garin and Bujadoux, 1922; Bannwarth, 1941; Dalsgaard-Nielsen and Kierkegaard, 1948; Schaltenbrand, 1962; Bammer and Schenk, 1965; Hörstrup and Ackermann, 1973). However, the discovery of the causative spirochete *Borrelia burgdorferi* in 1982 (Burgdorfer *et al.*, 1982; Benach *et al.*, 1983; Steere *et al.*, 1983), and the subsequent

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development of specific immunodiagnostic tests, have enlarged the clinical spectrum of neuroborreliosis, and allowed a better demarcation from other neurological disorders. Due to the increasing interest in this neurological disease during the past 10 yrs, many well-documented unusual and rare manifestations have been described (Midgard and Hofstad, 1987; Uldry *et al.*, 1987; May and Jabbari, 1990). However, many merely anecdotal and less firmly established descriptions of various nervous system disorders have also been attributed to Lyme borreliosis. The latter have usually been based on the presence of antibodies to *B. burgdorferi* in serum only. It has thus become difficult to determine from the literature not only the spectrum, but also the absolute and relative frequencies of the different manifestations of Lyme neuroborreliosis.

Previously reported series of patients with neuroborreliosis were collected at single clinical units, i.e. departments of neurology (Pachner and Steere, 1985; Sindic *et al.*, 1987; Kristoferitsch, 1989), infectious diseases (Stiernstedt, 1985) or paediatrics (Christen and Hanefeld, 1986; Naglo and Wide, 1989). Because our laboratory performed all Lyme-serology in Denmark it has been possible in this study to enrol patients referred from all branches of medicine and furthermore from a large and defined population. Advantages of this approach are: (i) the size of the cohort of patients; (ii) the width of the recruitment of patients from all medical disciplines; (iii) lack of diagnostic bias due to one or more investigator's view; (iv) that epidemiological data such as incidence have become available. Meanwhile broad awareness of this disease and the high number of serological tests performed at the present time make it likely that even less frequent and atypical neurological manifestations of Lyme borreliosis would be recognized.

Since 1985 we have collected clinical data and CSF/serum samples from all patients where the clinical condition, lymphocytic pleocytosis in CSF and/or intrathecal antibody examination pointed to the diagnosis of neuroborreliosis. The presence of specific local antibody synthesis within the CSF is currently the best indicator of neuroborreliosis (Stiernstedt *et al.*, 1985; Wilske *et al.*, 1986; Hansen and Lebech, 1991). Therefore in order to increase the certainty of the diagnosis and thus the conclusions of this study we have only included patients with a definite *B. burgdorferi* specific intrathecal antibody production. We believe that this study based on 187 prospectively collected patients reflects: (i) the main clinical and epidemiological features of neuroborreliosis in Denmark; (ii) that there are characteristic patterns of the clinical appearance of neuroborreliosis.

METHODS

Patient population

Patients in the study were collected prospectively over a 6 yr period from 1985 to 1990. They represent all patients recognized in Denmark with intrathecal *B. burgdorferi* specific antibody production, inflammatory CSF changes and active disease.

During the study period all Lyme-serology in Denmark (population 5.13 million) was performed in our laboratory at the Statens Seruminstitut, with one minor exception: from February 1, 1990 the county of Sønderjylland (population 250 132) performed its own serology. Thus data from this region are not included for the last 11 mths. From 1985 to 1990 the laboratory received 45 870 samples; 13% of them were CSF. The number of tests has steadily increased; in 1990 we analysed 13 581 samples, 1676 (12.3%) of these were CSF.

All patients had been admitted to a hospital and had at least one CSF examination. During the study period we recommended a diagnostic CSF analysis before treatment, and in order to follow the treatment response, a white blood cell (WBC) count and protein concentration in the CSF were measured 1–2 d after antibiotic therapy was completed and again 6 wks and/or 3 mths later. Complete hospital records, including all clinical and laboratory investigations were obtained on every patient. In March 1991 all patients received a questionnaire for us to obtain a final follow-up status. If further information was necessary, patients were contacted by telephone. Relevant data were extracted into a dataform which allowed us to prepare the data using DBase III plus. The study was approved by the Danish Ethical Committee.

Immunodiagnostic tests for B. burgdorferi specific antibodies

All serological tests used purified native *B. burgdorferi* flagella as test antigen (Hansen *et al.*, 1988, 1991). Specific IgG and IgM antibodies in serum were measured by an indirect and a μ-capture enzyme immunoassay (EIA), respectively; the diagnostic cut-off limit of these assays was adjusted to be 98% specific. Optical density (OD) values ≥ 0.160 for IgG and ≥ 0.4 for the IgM were regarded as positive. *Borrelia burgdorferi* specific intrathecal IgG and IgM production was measured by a highly sensitive γ- and μ-capture-EIA (Hansen and Lebech, 1991). This assay measures the proportion of specific antibodies to the total immunoglobulin in paired CSF and serum samples. An increased proportion of specific antibodies in CSF directly reflects intrathecal antibody synthesis. The test result was expressed as a specific capture antibody index OD-CSF/OD-serum × (OD-CSF − OD-serum). An index ≥ 0.3 indicates definite intrathecal *B. burgdorferi* specific antibody production (Hansen and Lebech, 1991). Patients included in this study had a *B. burgdorferi* specific capture antibody index ≥ 0.3 for IgM, IgG or both in either a first pretreatment sample ($n = 176$) or in the post-treatment sample ($n = 11$). Serological findings in serum and CSF of all patients are shown in Fig.1.

Statistics

All data are described in nonparametric terms and tests are specified in the text.

CASE HISTORIES ILLUSTRATING TYPICAL FEATURES OF THE COMMON COURSES OF NEUROBORRELIOSIS

Case 37: lymphocytic meningoradiculitis (Bannwarth's syndrome)

A 55-yr-old female was admitted to a department of neurology in October 1985 with severe back pain, facial diplegia and a sixth nerve palsy. Eight weeks before she had noticed a small circular erythema with a tick in the centre over her left hip bone. The erythema increased to a diameter of 30 cm within a few days and disappeared after 2 wks. Four weeks before admission she experienced severe burning pains in her lower back irradiating to the left flank and groin at night. After some days she also complained of constant severe pains between her shoulder blades and in the neck region. The pain was excruciating and worse at night. Three weeks later she developed a right incomplete facial palsy, 5 d later a left sixth nerve palsy and finally 1 d later a complete left facial palsy.

On admission, a CSF examination revealed 224 WBC/μl (98% mononuclear), a protein concentration of 2.0 g/l, an albumin ratio of 53.8×10^{-3} and increased *B. burgdorferi* specific antibody indices: IgG 16.5; IgM 5.2. *Borrelia burgdorferi* specific antibody levels in serum were increased (IgG OD = 0.770 and IgM OD = 0.580). She was treated with i.v. penicillin G for 10 d. The pain disappeared completely within 2−3 d. A CSF sample obtained 2 d after the end of therapy showed 43 WBC/μl, a protein concentration of 0.6 g/l and an albumin ratio of 15×10^{-3}. The facial diplegia recovered completely within 14 d and the sixth nerve palsy within 6 wks.

An EEG showed runs of 4−5 Hz slow waves mainly over the left temporal lobe. A third CSF sample 5 yrs later revealed 2 WBC/μl, a protein concentration of 0.5 g/l and no *B. burgdorferi* specific intrathecal antibody production.

Case 211: painful radiculitis without focal neurological signs

A 59-yr-old female was admitted to a surgical department in August 1990 with a 10 d history of severe deep burning pain in the right abdominal flank irradiating to the back and right thigh. A kidney stone was suspected but an i.v. pyelogram was normal. Over the following 2 mths she was readmitted to a department of internal medicine three times with continuous excruciating burning pains with pronounced nocturnal exacerbation. Three weeks before admission she had noticed a 10×15 cm large, well-demarcated, slightly painful non-vesicular erythema in the right abdominal flank. During her second admission she was treated with morphine, aciclovir and carbamazepine as a herpes zoster neuralgia was suspected. None

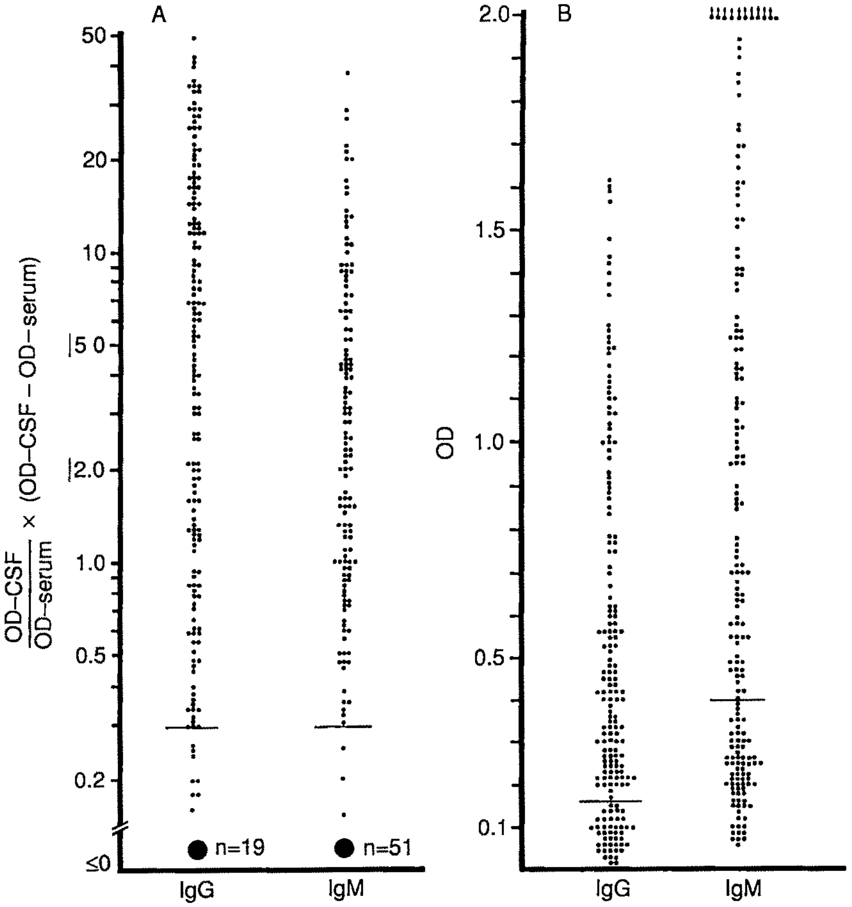

FIG. 1. A, *B. burgdorferi* specific intrathecal IgG and IgM antibody synthesis before therapy in 187 patients with neuroborreliosis. In 11 patients there was no specific IgG or IgM production in CSF before 10–12 d after the start of therapy. Specific antibody synthesis is expressed as an index: OD-CSF/OD-serum × (OD-CSF – OD-serum) (*see text*) Index values ≥0.3 indicate definite *B. burgdorferi* specific intrathecal antibody production ● indicates the specified number of patients with an antibody index ≤0 B, anti-*B. burgdorferi* IgG and IgM levels in serum of 187 patients with neuroborreliosis The horizontal lines mark the 98% specific cut-off limits.

of these drugs were effective. An increased level of *B. burgdorferi* specific antibodies in serum (IgG OD = 1.0 and IgM OD = 0.740) was found by her general practitioner leading to the suspicion of neuroborreliosis and to her final readmission. There was no headache and except for a slight bilateral papilloedema she showed no focal neurological signs. A cerebral CT was normal. A CSF examination revealed an opening pressure of 12 cm of CSF, 360 WBC/μl (98% mononuclear cells) and a protein concentration of 3.31 g/l. *Borrelia burgdorferi* specific intrathecal antibody indices were increased: IgG 6.9; IgM 7.2. She was treated with i.v. penicillin G for 2 wks. Within days the radicular pain disappeared. A CSF sample obtained 2 d after therapy had been completed showed 2 WBC/μl and a protein concentration of 1.47 g/l.

Case 36. radiculomyelitis

In September 1985 a 65-yr-old female noticed a 5 × 10 cm macular erythema on her right shoulder lasting 1 wk. Two weeks later she experienced severe burning pain in her lower neck and entire shoulder girdle, paraesthesiae in both arms and 1 wk later increasing proximal and distal paresis first of the right arm and 2 d later of the left arm, but less pronounced. On admission to a department of neurology she showed bilateral infranuclear paresis of shoulder, arm and hand muscles, attenuated biceps and triceps reflexes, brisk knee and ankle reflexes, bilateral ankle clonus and a left Babinski's sign. A cervical myelogram was unremarkable. Routine CSF examination in connection with the myelogram revealed an increased cell count. A second CSF sample showed pleocytosis with 146 WBC/μl, consisting of lymphocytes but also many plasma cells. The spinal protein concentration was 1.54 g/l, the albumin ratio 23.4×10^{-3}, the IgG index 1.54 and *B. burgdorferi* specific intrathecal antibody indices: IgG 9.5; IgM 10.3. *Borrelia burgdorferi* specific antibody levels in serum were increased (IgG OD = 0.3 and IgM OD = 0.64, respectively). After a course of 10 d of i.v. penicillin the pain disappeared. A third CSF examination 2 d after treatment was completed showed 74 WBC/μl, a protein concentration of 0.94 g/l and albumin ratio 13.4×10^{-3}. The arm paresis recovered completely within 2 mths. A CSF examination 6 wks later revealed 8 WBC/μl, and a spinal protein of 0.67 g/l.

Her EEG was normal. Electromyography of five shoulder and upper limb muscles showed neurogenic involvement. The median nerves showed normal distal motor latencies and normal motor and sensory nerve conduction. Normal F-wave latencies were recorded upon stimulation of the median and ulnar nerves. Sensory evoked potential examination of both median nerves revealed increased central conduction times (C7-cortex) of 33% (left) and 40% (right); regarding the tibial nerves only results from stimulation of the left nerve were abnormal with an 38% increased central conduction time (Th12-cortex).

Case 171: monosymptomatic facial palsy in a child

A 5-yr-old boy was admitted to a department of paediatrics in October 1989 with a complete left facial palsy. There was no fever, headache or meningeal signs. Three weeks before he had a 1 cm broad migrating erythema expanding from the left forehead to the left ear, transient pain around the left shoulder and behind the left ear An otoneurological examination showed loss of the left stapedial reflex and normal taste and lacrimation. Examination of the CSF 1 wk after admission revealed 150 WBC/μl (90% mononuclear), protein concentration 0.61 g/l and increased *B. burgdorferi* specific intrathecal antibody indices: IgG 12.4; IgM 64.3. Anti-*B. burgdorferi* IgG level in serum was normal (OD = 0.090) and IgM was slightly increased (OD = 0.430). He received i.v. penicillin G for 10 d. Examination of CSF the day after the last penicillin dose, showed 42 WBC/μl and a protein concentration of 0.3 g/l. The facial palsy recovered completely.

Case 256: subacute lymphocytic meningitis in a child

A 9-yr-old boy was admitted to a department of pediatrics in November 1990. The disease had started in July 1990 with a 14 d period of influenza-like symptoms. During the following 4 mths he constantly complained of general symptoms, nausea, moderate to severe headache, intermittent slight back pain and loss of appetite. He had an intermittent fever (37.5–39°C) and there was a 3 kg weight loss. The intensity of his symptoms varied markedly from day to day. One month before admission he had generalized tender lymph node swelling. On admission he showed no meningeal signs and physical and routine laboratory examinations were normal. A CSF examination 2 wks after admission revealed 148 WBC/μl, predominantly lymphocytes, a protein concentration of 0.88 g/l and increased *B. burgdorferi* specific intrathecal antibody indices: IgG 5.5; IgM 0.6. Anti-*B. burgdorferi* antibody levels in serum were increased (IgG OD = 0.8 and IgM OD = 1.49). He received i.v. penicillin G for 10 d and recovered completely within days. A CSF examination 4 wks after therapy showed six WBC/μl and a protein concentration of 0.4 g/l.

Case 78: chronic meningitis in an adult

A 61-yr-old female was admitted to a department of internal medicine in November 1986 with a 5 wk history of headache, intense pain in the neck and shoulder girdle, dizziness, nausea, vomiting, weight loss, fatigue and fever (38–38.5°C). Physical and routine laboratory examinations were normal. The back pain gradually diminished, while all other symptoms persisted. Her final weight loss was 12 kg. Repeated CSF examinations showed lymphocytic pleocytosis and increased protein concentrations. A CSF sample, obtained in February 1987, was subjected to syphilis serology and showed a positive Fluorescent Treponema Antibody-absorbance (FTA-abs) reactivity (2+), but a negative cardiolipin test (Wassermann). This serological profile led to the investigation for neuroborreliosis which was confirmed on the next CSF sample which showed increased *B. burgdorferi* specific intrathecal antibody indices: IgG 8.8; IgM 1.9. In serum only the *B. burgdorferi* specific IgG level was increased (OD = 0.6). The patient received i.v. penicillin

G for 18 d during March. Her condition improved significantly within 1–2 wks, followed by complete recovery within 6 mths.

Cerebrospinal fluid WBC/µl (1) and protein concentration g/l (2): Case 78

	Dec. 18, 1986	*Jan. 12, 1987*	*Feb. 3, 1987*	*Mar. 5, 1987*	*Apr. 29, 1987*	*Aug. 26, 1987*
(1)	685	720	231	309	100	8
(2)	3.6	3.96	3.8	3.7	1.5	0.77

Audiometry and caloric test were normal. Her EEG showed 2–5 Hz activity with a moderately increased amplitude most pronounced over the left temporal lobe.

Case 129: chronic progressive encephalo-myelitis

A 54-yr-old female was admitted to a department of neurology in March 1988 with a progressive ataxic gait disturbance. She had been healthy until November 1985 when she experienced a 2–3 mths period with intense pain in her neck and shoulder girdle. She continued to have headaches, dizziness and general symptoms. She was admitted for the first time in February 1986. Her clinical examination was normal apart from a right Babinski's sign; a CT scanning of the brain was normal and EEG showed 4–6 Hz activity over her left temporal lobe. A caloric test was normal. Audiometry showed a slight, predominantly left-sided sensorineural hearing loss. Several months later she felt a gradual onset of a slowly progressive impairment of gait, progressive loss of hearing and finally also an altered pattern of micturition. In January 1988 audiometry revealed a severe sensorineural hearing loss involving all but especially high frequencies; her threshold of intelligibility was 40 dB bilateral and the discrimination was reduced to 95% (right) and 90% (left). She was readmitted in March 1988 where she presented with signs of a moderate, mainly right-sided spastic paraparesis. Her gait was broad and ataxic. A CT scan of the brain showed periventricular lucencies and a hypodense lesion in the left capsula interna. Her EEG was unchanged. Visual evoked potential examination was normal; auditory evoked potential examination revealed an increased latency to peak numbers 4, 5, 6 on the left side. Somatosensory evoked potentials from the median nerves were normal. Stimulation of left and right tibial nerves showed a bilateral prolonged central conduction time of 46% and 23%, respectively. Cystometry revealed signs of a spastic bladder. The tentative diagnosis was a nonremitting form of multiple sclerosis. Repeated CSF examinations performed in September 1988, however, showed an unexpected lymphocytic pleocytosis, an increased protein concentration, a decreased CSF/blood glucose ratio of 1.3/5.4 (mmol/l) and oligoclonal IgG. A thoracolumbar myelogram was normal. *Borrelia burgdorferi* specific intrathecal antibody indices were increased: IgG 4.9; IgM 0. *Borrelia burgdorferi* specific IgG and IgM levels in serum were increased to an OD of 1.250 and 0.7, respectively. She was treated with i.v. penicillin October 4–14. When she was readmitted in November 1988 and April 1989 she had no general symptoms and no headache; she still had a Babinski's sign, but no paresis and her gait control had improved significantly. Her hearing gradually became normal and she could dispense with her hearing aid. Audiometry (March 25, 1991) showed an improvement as discrimination was normal and the threshold of intelligibility had improved to 10 dB (right) and 20 dB (left).

Cerebrospinal fluid WBC/µl (1) and protein concentration g/l (2): Case 129

	Sep. 14, 1988	*Sep. 19, 1988*	*Sep. 28, 1988*	*Oct. 14, 1988*	*Nov. 25, 1989*	*Apr. 5, 1990*
(1)	180	200	177	97	21	2
(2)	3.2	2.8	3.0	1.8	0.7	0.4

RESULTS

Demographical and epidemiological data

A total of 187 patients was included, 112 males and 75 females (sex ratio m/f = 1.5). Their age (median 49 yrs, range 4–86 yrs) followed a bimodal distribution (Fig. 2) with two peaks of incidence, one in childhood and one in the 50–60 yr range. The

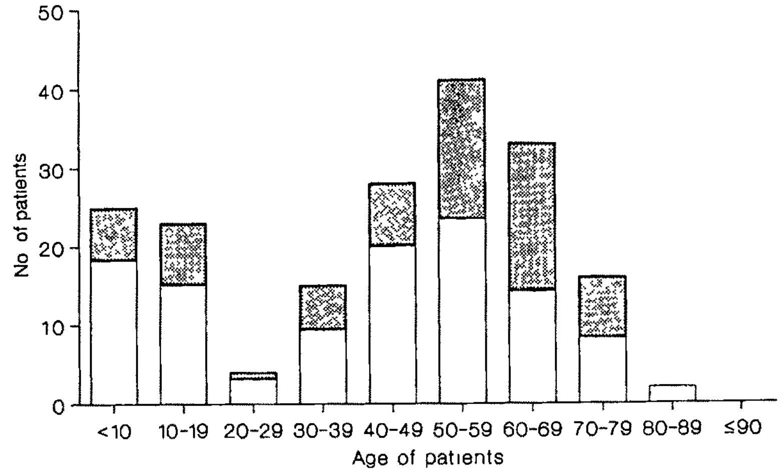

FIG. 2 Age and sex distribution of 187 prospectively collected patients with neuroborreliosis; the shaded area of the bars indicate the proportion of females Except one child of 4 yrs, all patients in the first decade were ≥5 yrs, thus yielding the highest age corrected incidence of neuroborreliosis.

annual number of patients increased steadily from 23 in 1985 to 47 in 1990. The overall incidence in Denmark in 1990 was 9.2/million, being highest on Funen with 24.1/million and in rural areas of Zealand with 15.5/million, and lowest in Jutland with 5.7/million. In five cases the infection was not acquired in Denmark. Twice, two members of the same household had Lyme borreliosis within the same season. Otherwise no clustering of cases within the same community was observed. Six patients were professional gardeners. None were forestry workers. Regarding other current or previous diseases, three patients had diabetes mellitus, eight had malignancies and two received steroid treatment. The diagnosis of neuroborreliosis was most commonly made in departments of neurology (46% of the cases), followed by departments of internal medicine (26%), pediatrics (18%), infectious diseases (4%), otolaryngology (3%), rheumatology (2%) and neurosurgery (1%).

Onset of neuroborreliosis was defined as onset of neurological symptoms, e.g. radicular pains. As shown in Fig. 3 the onset of neuroborreliosis occurred mainly from June to December. The time from onset to the date of diagnosis ranged from 4 d to 6 yrs with a median of 25 d.

Tick bite and erythema migrans

A previous tick bite was recalled by 48 patients (26%). According to observations from 24 patients the latency between a tick bite and erythema migrans was 1–16 d (median 3 d). An erythema migrans was noticed by 86 patients (46%), whereas the erythema migrans or signs reminiscent of it were observed in only 29 patients at admission. The anatomical location of the tick bite and erythema migrans is shown in Table 1. Based on 55 patients the erythema migrans lasted from 6 d to 75 d (median 20 d). Multiple erythema migrans were not reported. Seventeen patients had sought

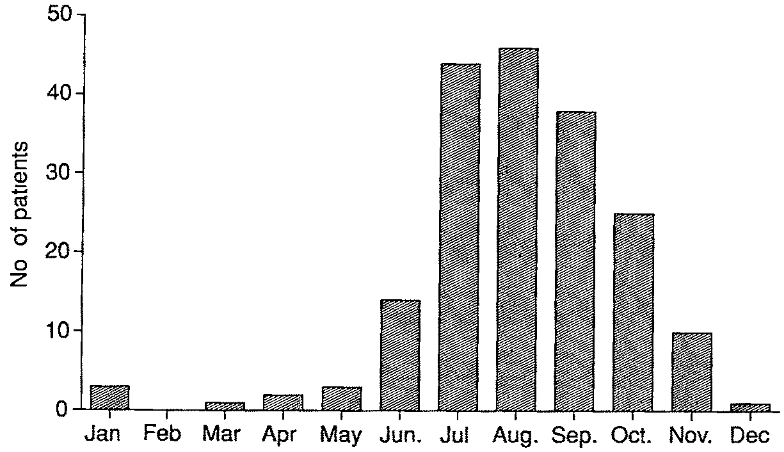

FIG. 3. Time of onset of neurological symptoms in 187 patients with neuroborreliosis

TABLE 1 TICK BITE AND ERYTHEMA MIGRANS IN 187 PATIENTS WITH NEUROBORRELIOSIS

Tick bite (n = 48)	Head and neck	12%
	Trunk	23%
	Upper limbs	21%
	Lower limbs	44%
Erythema migrans (n = 86)	Head and neck	12%
	Trunk	30%
	Upper limbs	20%
	Lower limbs	38%

medical attention for their erythema migrans; in 11 patients an erythema migrans was diagnosed but six were treated either with oral penicillin V in insufficient dosage (n = 3; 0.5 million units three times daily for less than 10 d) or with oral erythromycin (n = 3; 1 g twice daily for 10 d); and five patients whose symptoms of neuroborreliosis were already present but not recognized were treated with oral penicillin only. Of the remaining six patients four were left without a diagnosis and two were misdiagnosed as either erysipelas or a mycosis.

Clinical features of neuroborreliosis

According to the main clinical symptoms and signs, we classified the 187 patients into six main groups as shown in Table 2, being aware that some patients had overlapping features.

Painful sensory radiculitis

Radiculitic pain was a major symptom and present in 160 patients (86%), beginning 5–90 d (median 19 d) after the erythema migrans. The intensity of radicular pain increased significantly with the age of the individual (Fig. 4). The onset was in most

TABLE 2 CLASSIFICATION OF 187 PATIENTS WITH NEUROBORRELIOSIS ACCORDING TO THE MAIN CLINICAL FEATURES AT THE TIME OF DIAGNOSIS

	No. of patients	*Percentage*	*Reference case no*
Second stage (early) neuroborreliosis			
LMR with mononeuritis multiplex (Bannwarth's syndrome)	114	61.0	37; 171
LMR without paresis (radiculitis pain syndrome only)	46	24.6	211
Subacute lymphocytic meningitis (without paresis, without radicular pains)	9	4.8	256
Myelo-meningoradiculitis*	7	3.7	36
Third stage late neuroborreliosis			
Chronic lymphocytic meningitis (paresis, disease duration ≥6 mths)	3	1.6	78
Chronic progressive encephalomyelitis	8	4.3	129

LMR = lymphocytic meningoradiculitis; *including the only patient with clinical signs of encephalitis

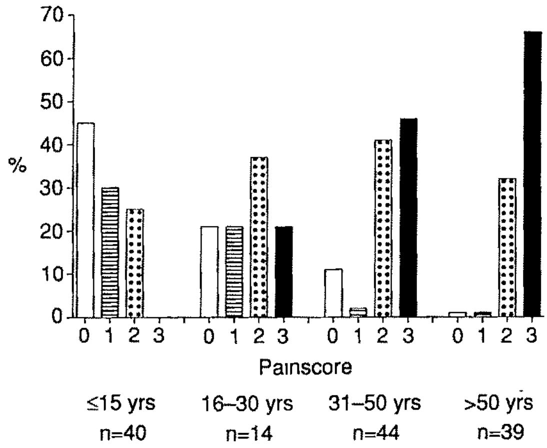

FIG. 4 Intensity of radicular pain and age in 187 patients with neuroborreliosis. The intensity of pain was graded: 0 = no radicular pain, 1 = slight; 2 = moderate; 3 = severe. Comparing the frequency of a pain score 1 and 2 versus 3 and 4 in the four age groups revealed a significant correlation of pain and age (χ^2 test for trend χ = 83.45, f = 1; $P<0.000001$).

instances subacute, occurring overnight. Pain often began in the region of a previous erythema migrans, then migrated and finally became most pronounced axially in the back typically between the shoulder blades but also in the neck or lumbar region. Some patients had migrating pain almost over the whole body. The pain was described as being of a type never experienced before and was easily distinguished from ordinary back pains. The intensity of the pain often varied from day to day and typically showed severe nocturnal exacerbations (n = 68). Pain was described by most patients as severe, burning, deep and/or superficial as if located in the skin, and often accompanied by patchy areas of unpleasant hyper- and dysaesthesiae (n = 63). Occasionally a belt-like sensation around the trunk was described. Severe pain was refractory to morphine. Forty-six patients (25%) had radiculitic pain only and never developed focal motor signs. In 54 patients the sensory radiculitis either diminished or disappeared even before therapy after 2–16 wks.

Before the final diagnosis of neuroborreliosis was established, the painful condition was often thought to be due to other diseases, e.g. herpes zoster neuralgia, cervical or lumbar nerve root compression, facet syndrome, brachial plexus neuropathy, polymyalgia rheumatica, myocardial infarction or kidney concrements. In one female a cholecystectomy was performed, three patients underwent a biopsy of the temporal artery, three an i.v. pyelogram, seven a bone scintigraphy and 17 a myelogram, before the final diagnosis. Routine examination of the CSF obtained during the myelography unexpectedly revealed pleocytosis leading to the correct diagnosis.

Some patients presented with an agitated mental state. This was probably due to a severe pain condition and deprivation of sleep for days to weeks. Thirteen patients were initially suspected of being hysterical and several were examined by a psychiatrist because of the apparent disproportion between their dramatic complaints and the lack of signs of disease.

Headache and meningeal signs

Headache was reported by 80 patients (43%); in 14 the headache was mild, in 53 moderate and in 13 patients severe. The intensity varied often considerably and headache was in most patients not a dominating symptom. Only a few patients experienced unilateral periauricular pains before developing a facial palsy.

A prominent headache without radicular pain or paresis was only seen in children, apart from three women aged 37–64 yrs. On the other hand, nine adults developed chronic headache often with significant general symptoms, such as weight loss and nausea of ≥6 mths duration after a previous period of pain compatible with a radiculitis. Six of these nine patients were diagnosed when they developed signs of chronic encephalomyelitis. The remaining three were diagnosed as having a chronic lymphocytic meningitis 6–12 mths after onset, mainly presenting as chronically diseased with headache but without meningeal or focal motor signs (Case 78).

Meningeal signs such as neck stiffness were reported in only 19 patients; 15 of these were aged ≤15 yrs. Grading the neck stiffness showed 10 cases to be borderline to mild rigidity, and nine to have definite neck stiffness, but never as pronounced as in purulent meningitis.

Focal neurological signs in second stage neuroborreliosis

A total of 120 of the patients had peripheral motor paresis; 86 had only cranial nerve involvement, 22 had only limb paresis and 12 had cranial nerve and limb paresis. Seven patients showed clinical signs of acute radiculomyelitis. Paresis occurred 1–99 d (median 15 d) after onset of pains.

Cranial neuropathy. The facial nerve was most frequently affected; 95 patients (51%) had a facial palsy which was unilateral in 65 and bilateral in 30 patients. Seventeen (26%) of the unilateral paresis were complete. In patients with facial diplegia, one side was complete in 13 and bilaterally complete in seven. Facial diplegia always developed consecutively with an interval of 1–23 d (median 6 d) between the two sides; sometimes the second paresis even began after remission of the first one. Several patients with facial paresis reported numbness of the ipsilateral face, but signs of trigeminal nerve involvement were not reported. Abducens palsy was reported in nine patients, in one it was bilateral. In three patients the sixth nerve palsy was the only focal motor sign. Unilateral oculomotor nerve palsy occurred only once. No other cranial nerve paresis was reported.

Limb and trunk paresis without signs of myelitis. Twelve patients had paresis in the upper extremities, in eight it was unilateral and in four bilateral. Sixteeen patients had leg paresis, in 10 it was unilateral and in six bilateral. When bilateral, the paresis was always asymmetrical in onset and severity. Two of the patients presented with simultaneous arm and leg paresis and a further two had paresis of the abdominal wall. The distribution of paretic muscles usually indicated involvement of multiple nerve roots. Tendon reflexes were attenuated or absent only corresponding to the muscle weakness.

Central nervous system involvement. In the second stage, seven patients had signs of myeloradiculitis indicated by Babinski's sign (n = 6), transversal impairment of sensation (n = 2) and supranuclear bladder paresis (n = 1). A somatosensory evoked potential (SEP) examination was performed on five of these patients and revealed a prolonged central conduction time in three. Six of the patients with signs of myelitis had moderate to severe limb paresis where in all but one case the limbs were flaccid with attenuated or absent tendon reflexes; one patient also had an infranuclear bladder and sphincter paresis as well. Electromyography (EMG) in three patients indicated a peripheral axonal involvement (Case 36). A 9-yr-old child with an encephalo-myelo-radiculitis was periodically somnolent. Otherwise none of the patients had signs of acute encephalitis with impairment of consciousness and/or seizures.

Third stage chronic progressive encephalomyelitis

Eight patients, one or two per year, were diagnosed as third stage chronic progressive encephalomyelitis. The duration of their disease ranged from 6 mths to 6 yrs (median 2 yrs). Most of them were admitted to hopsital due to a slowly progressive myelopathy beginning with an ataxic gait (n = 8), spastic bladder paresis (n = 5) and finally a slight to moderate spastic para- or tetraparesis (n = 8). A characteristic finding was a progressive sensorineural hearing loss (n = 6). Headache and mild to moderate general symptoms were continuously present in six patients.

Neuroborreliosis in children

There were 40 children (≤15 yrs) among the 187 patients. Evaluation of their symptoms and signs revealed marked differences compared with adults. The median disease duration in children before diagnosis was 18 d (3 d to 18 wks). Whereas radicular pain was significantly less common (n = 22) and if present less severe (Fig. 4), headache was considerably more frequently reported (n = 28, 70%) than in adults (n = 51, 35%) ($P < 0.001$; Fishers two-sided exact test). Clinical signs of meningitis were reported in 15 children (38%) but only in four adults (3%) ($P < 0.001$). Fever at admission (≥38°C) occurred in 40% of the children but only in 10% of adults ($P < 0.001$). The frequency of paresis in children and adults was similar, 68% and 69%, respectively. The occurrence of a monosymptomatic paresis (usually facial paresis) without concomitant pain, fever or neck stiffness was only reported in children (n = 7, Case 171) except for a 32-yr-old male with an abducens palsy. Twenty-six children had a facial palsy which was bilateral in only three cases. Facial diplegia was thus less frequent than in adults ($P < 0.014$). Only one 9-yr-old boy had CNS involvement with somnolence and acute myelitis. Because of the less pronounced painful radiculitis, neuroborreliosis in children is often less dramatic and may be an insidious condition with fluctuating headache, loss of appetite, weight loss, intermittent low grade fever, sometimes slight meningeal signs, but no focal neurological signs, lasting for weeks to months (Case 256).

Extraneurologic findings

Two patients had a monarthritis with swelling of a knee and an ankle, respectively, during hospitalization. In 10 other patients oligoarthralgias without signs of arthritis were reported. It was common for radicular pain, when located in limbs, to be initially referred to joints by patients and doctors.

None of the patients had cardiac symptoms. In 148 patients an ECG was obtained; typical signs of Lyme carditis (van der Linde *et al.*, 1990) with A-V conduction bloc were not reported. A 24-yr-old patient with intense radicular pains developed severe hypertension during hospitalization. Two months after antibiotic therapy, antihypertensive treatment was stopped and the patient remained normotensive.

Topographical association of paresis and erythema migrans

There was a significant topographical association of the site of erythema migrans and the site of the first peripheral paresis. If patients with only an erythema migrans in one extremity and a subsequent paresis are considered (Table 3), the first paresis is significantly more common in the limb where the erythema had been than would be expected if only haematogenous spread was responsible for the dissemination to the nervous system. Of eight patients with a unilateral erythema migrans in the face or neck

TABLE 3 TOPOGRAPHICAL ASSOCIATION OF ERYTHEMA MIGRANS AND THE FIRST MOTOR PARESIS

	No. of patients with (1) motor paresis in		
Patients with EM + paresis	*Same extremity*	*Opposite extremity*	*Other locations*
Right arm (n = 5)	1	0	4 (VII)
Left arm (n = 8)	5	0	3 (VII)
Right leg (n = 9)	4	0	5 (4 VII, 1 arm)
Left leg (n = 10)	3	0	7 (5 VII, 2 arms)
n = 32	13*	0*	19

Based on the binomial distribution the P value for this outcome is (one-sided test) 0.0001, EM = erythema migrans; only patients with an erythema migrans on an extremity were included; VII = facial palsy.

region, six developed a paresis and in every case this was a facial palsy occurring on the same side as the previous erythema migrans (Case 171).

The evolution of neuroborreliosis

Symptoms and signs of neuroborreliosis usually developed in a characteristic sequence: tick bite, followed by erythema migrans, then sensory radiculitis and finally focal motor signs. The median time and range which evolved between the different steps in individual patients are summarized in Table 4. Exceptions from this scheme were: one patient who developed paresis 5 d before onset of pain and eight patients with monosymptomatic paresis. In 46 patients the sensory radiculitis was not followed by a subsequent paresis.

Not included in Table 4 is the evolution of chronic encephalomyelitis. After an episode compatible with a meningoradiculitis six of these patients continued to have general symptoms and/or headache, until 2 mths to 2 yrs later when signs of a chronic progressive encephalomyelitis began (Case 129). In two patients, a chronic myelitis developed without apparent previous disease.

TABLE 4. EVOLUTION OF SECOND STAGE NEUROBORRELIOSIS

	Latency in days		*No of observations*
	Range	*Median*	
Tickbite			
↓	0–16	3	24
Erythema migrans			
↓	5–90	19	77
Sensory radiculitis			
↓	1–99	15	97
First paresis			
↓	1–30	7	40
Last paresis			

Routine laboratory blood examinations

Routine laboratory investigations (Table 5) were usually unremarkable and of no diagnostic value.

Cerebrospinal fluid changes

The CSF cell count, protein concentration, albumin ratio and IgG index before therapy are summarized in Table 6. All but nine patients had prominent lymphocytic pleocytosis $>20/\mu l$. A relatively low cell count (4–20 WBC/μl) in eight patients could be attributable to (i) pretreatment with oral antibiotics before admission ($n = 2$); (ii) pretreatment with steroids for 3 wks ($n = 1$); (iii) spontaneous remission due to a disease duration ≥ 3 mths ($n = 5$). Apart from patients with a chronic course, the level of CSF pleocytosis usually decreased even before therapy if the disease duration exceeded 3 mths (Fig 5), whereas the protein concentration decreased more slowly. The CSF/blood glucose ratio was examined in 166 patients; a significantly decreased ratio ($\leq$ 0.3) was only seen in seven patients; six of these had chronic progressive encephalomyelitis and one chronic meningitis of 6 mths duration (e.g. Cases 78, 129).

TABLE 5. ROUTINE LABORATORY BLOOD TESTS

Test	*No. of patients tested*	*No. of patients with value above the indicated reference limit*		*Median value of increased values*	*Highest level*
Erythrocyte sedimentation rate	159	20	≥20 mm/h	27	71
Peripheral blood leucocyte count	178*	22	≥10.0×10^9/l	11.7	16.7
Amino-transferases (ALAT/ASAT)	101	5	>40 U/l	95	114
Alkaline phosphatase	101	12**	>275 U/l	411	680

*Differential count performed on 141 patients: normal n = 120; relative lymphopenia (10–20%) n = 21, **including eight children ≤13 yrs.

TABLE 6. CEREBROSPINAL FLUID FINDINGS PRE- AND POST-TREATMENT

	Pretreatment median time after onset 25 d	*Median time for CSF sampling after start of therapy*		
		12 d	*11.5 wks*	*35 wks*
	n = 187	n = 112	n = 72	n = 16
Cell count per μl (<3)	160 (4–1000)	42 (1–289)	6 (1–100)	4 (1–18)
Protein conc. g/l (≤0.5)	1.13 (0.2–12.0)	0.68 (0.12–3.1)	0.44 (0.14–1.52)	0.51 (0.2–1.56)
	n = 102	n = 54	n = 39	n = 4
Albumin ratio ($\times10^{-3}$) ($<10\times10^{-3}$)	19.7 (3.3–68.9)	11.2 (2.2–41)	6.5 (2.3–20.9)	6.4 (3.6–12.8)
IgG index (<0.7)	0.84 (0.4–2.43)	0.77 (0.31–2.01)	0.64 (0.15–2.07)	0.55 (0.53–1.23)

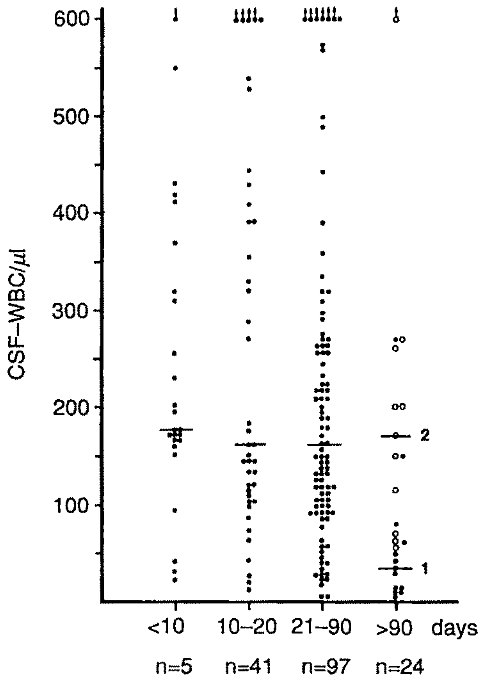

FIG. 5. The level of CSF pleocytosis before therapy in relation to the disease duration. Open circles indicate patients with chronic disease (≥6 mths of disease duration). The horizontal bars indicate median values; the median cell count in patients with a disease duration of >3 and <6 mths (1) was 35 WBC/μl, whereas in patients with chronic disease it was 170 WBC/μl (2).

The CSF opening pressure was measured in 73 patients. A slightly increased pressure between 20 cm and 29 cm (median 24 cm) was reported in 15 patients.

Antibodies to B. burgdorferi in serum and CSF

At the time of diagnosis 44 patients (24%) had an insignificant level of specific IgG antibodies in serum (Fig. 1); the median disease duration in these patients was 19 d (6–54 d). Thirty of these patients, however, had an increased specific IgM level and thus only 14 had neither specific IgG nor IgM in their first serum sample. Regarding pretreatment CSF samples (Fig. 1), 27 patients (14%) revealed no specific intrathecal IgG synthesis, their median disease duration being 11 d (4–54 d); however, 16 of them (59%) showed a specific local IgM production in this first CSF sample. In 11 patients [6%; median disease duration 10 d (4–30 d)], with neither specific IgG nor IgM in the CSF before therapy, a specific intrathecal antibody production was found in a second sample taken immediately after therapy. All patients were negative in a nontreponemal test for syphilis (Wassermann reaction).

Audiometry and vestibular function

Except for six patients with third stage chronic neuroborreliosis none complained of impaired hearing. Audiometry was performed in 51 patients. A significant sensorineural hearing loss was found in all six patients with chronic neuroborreliosis who experienced a progressive hearing loss within 1–2 yrs of onset (Case 129). The audiometry was uneventful in the remaining 45 patients, of whom 15 had a bilateral and 16 a unilateral facial palsy.

None of the patients experienced symptoms compatible with neuronitis vestibularis. Caloric stimulation was performed in 30 patients; 12 had a unilateral and nine a bilateral facial palsy. Only two patients' tests were abnormal. One patient with a facial diplegia showed an asymptomatic unilateral vestibular paresis. Another patient with a chronic encephalomyelitis and a severe bilateral sensorineural hearing loss showed pendular nystagmus and a caloric test with directional preponderance.

Fundoscopy

Fundoscopy was performed in 143 patients. Abnormal findings were reported in five patients; two showed unilateral and three slight bilateral papilloedema. In three of the five patients, CSF opening pressure was measured and was normal. Visual acuity was normal in all patients. None had symptoms compatible with retrobulbar neuritis.

Electroencephalography

An EEG was recorded before treatment in 60 patients. It was normal in 28 and slightly abnormal with unspecific changes in 13. Significant changes were found in 19 patients (32%) and consisted of frontotemporal runs of slow wave activity with a shifting side preponderance. The slow wave frequencies were 1.5–3 Hz in 10 patients and 3–5 Hz in nine patients. Sharp waves were not uncommon. The EEG findings in Cases 37, 78 and 129 are representative.

Electromyography and nerve conduction studies

Electromyography and nerve conduction studies were performed in six patients with limb paresis 2–12 wks after onset and in seven patients with facial palsies 4 d to 6 wks after onset.

In the six patients with limb paresis EMG revealed (i) marked loss of motor units on maximal volition (n = 6); (ii) fibrillation potentials (n = 4); (iii) positive sharpe-waves (n = 2); (iv) increased duration of motor unit potentials (n = 4); (v) increased occurrence of polyphasic potentials (n = 5). Distal motor latencies were increased in only two patients, once in the femoral nerve (45%) and once in the peroneal nerve (32%). Electromyography in the seven patients with facial palsies showed (i) severely prolonged (>50%) distal latencies along the facial nerve (n = 4); (ii) marked loss of motor units on maximal volition (n = 6); (iii) fibrillation potentials in facial muscles (n = 3).

Peripheral motor and/or sensory nerve conduction was studied along the following nerves: (i) median and sural (n = 6); (ii) median and ulnar (n = 1); (iii) femoral and tibial (n = 3); (iv) peroneal and sural (n = 2); (v) sural only (n = 1). Findings were normal except in two patients: one patient with a quadriceps paresis showed a decreased amplitude of the muscle action potential upon stimulation of the femoral nerve and the other a decreased peroneal motor nerve conduction velocity. F-wave latencies were measured in five patients and were always within normal limits.

Cerebral computerized tomography (CT)

A cerebral CT scan was performed in 51 patients. In all but two patients contrast enhancement was used. Only three patients with a chronic progressive encephalomyelitis had abnormal scans. The CT showed multiple hypodense white matter lesions mainly located adjacent to the anterior horns but in one case also in the capsula interna. The radiological diagnosis was a leukoencephalopathy.

Antibiotic therapy and treatment response

Only one patient was not treated with antibiotics. Highdose i.v. penicillin G (20 million units per day for 10 d) or a proportionally reduced dosage for children, was the preferred treatment and used in 170 patients (91%). Other regimens were 10 d of ceftriaxone 2 g i.v. once a day (n = 5); i.v. penicillin G for 3 d followed by ceftriaxone for 7 d (n = 2); oral doxycycline, 400 mg per day (n = 1); oral chloramphenicol, 0.5 g four times daily (n = 3); cefotaxim or cefuroxim 2 g three times daily (n = 3). Two patients received insufficient dosage of either oral penicillin or i.v. penicillin for only 7 days.

A Jarisch Herxheimer reaction was never reported. In six patients a peripheral paresis developed after the start of therapy, usually within 1–2 d and never later than 5 d. The common and most dramatic response to treatment was the relief of radicular pain and headache. In some, the pain had disappeared completely by 1–4 d, whereas in the majority the pain intensity was reduced markedly by 1–4 d and pains were then present intermittently for 1–2 wks. Intermittent radicular pain and dysaesthesiae, usually lasting 1–2 mths but occasionally 6–12 mths after therapy, was reported by approximately 20% of the patients. Recovery from general symptoms and fatigue often took months. In patients with longlasting meningitis and with chronic encephalomyelitis the onset of improvement after therapy was slower but nevertheless marked.

Cerebrospinal fluid investigations after therapy

A lumbar puncture was performed in 112 patients shortly after therapy. The CSF showed a marked reduction in cell count, protein concentration and albumin ratio (Table 6), indicating a rapidly declining inflammation in the meninges. Only in patients with chronic meningitis and encephalomyelitis was the initial reduction in these variables less prominent 1–2 d after therapy, being finally achieved within months (Cases 78, 129). Examination of CSF 3 mths and 9 mths after therapy (Table 6) confirmed the steady and continuous decline of inflammation. Unchanged or increasing CSF inflammation was never observed.

Recovery of focal neurological signs

After therapy the patients were followed up by the hospital for 1–66 mths (median 3 mths). The number of patients with a permanent paresis and its relation to the initial type of paresis is summarized in Table 7. The outcome of facial palsies in children seems more favourable than in adults, since only two with a unilateral and two with a bilateral paresis had a slight unilateral residual paresis. Of 48 patients with an incomplete unilateral facial palsy, four had minor sequelae, whereas of 17 patients with an initially complete palsy, six had a minor and two a severe residual paresis. Regarding facial palsy, 82% showed a first sign of recovery within 1 mth after onset. All patients with a severe residual paresis showed a first sign of recovery more than 3 mths after onset. Patients with limb paresis and signs of myelitis more frequently had severe sequelae (Table 7). In all eight patients with chronic progressive encephalomyelitis, therapy stopped further progression and seven patients experienced significant improvement of their paraparesis and gait control. One patient with a history of benign intracranial hypertension 5 yrs before became severely disabled probably as a result of an episode of brain herniation triggered by the meningitis.

TABLE 7 PERMANENT MOTOR SEQUELAE

	No of patients with initial paresis	*No. of patients with*	
		Slight sequelae (%)	*Severe sequelae (%)*
Facial paresis			
Unilateral	65	10 (15)	2 (3)
Bilateral	30	11 (37)	2 (7)
Sixth nerve palsy	9	2 (22)	0
Limb paresis arm	12	1 (8)	0
leg	16	5 (31)	1
Limb paresis with signs of myelitis	6	0	4 (67)
Chronic encephalomyelitis	8	3 (38)	5 (62)

Follow-up

A final follow-up was carried out after 4–72 mths (median 33 mths) in all patients except two who had died from cancer. Fourteen patients reported sensory complaints, most frequently distal paraesthesiae and less frequently patchy areas of dysaesthesiae or hyperaesthesiae; three patients still had episodes of radicular pain. One patient with a previous chronic meningitis suffered from chronic polyarthralgias without signs of arthritis. Her condition did not improve after retreatment with i.v. penicillin or ceftriaxone. One patient was reinfected and experienced a new episode of neuroborreliosis after 4 yrs. Otherwise none of the previous patients developed further progression, relapses or complications attributable to neuroborreliosis. Of the 185 patients still living, 175 including all the children resumed their normal daily activities (work, education, etc.) without limitations; five patients had a reduced working capacity and five had retired because of disablement following neuroborreliosis.

DISCUSSION

The triad of Bannwarth's syndrome consisting of painful radiculitis, a mainly cranial mononeuritis multiplex and CSF lymphocytic pleocytosis still reflects the basic pattern in neuroborreliosis (Table 2). However, the increasing awareness of this infection and the introduction of reliable tests for *B. burgdorferi* specific intrathecal antibody synthesis have enlarged the clinical spectrum. This has primarily led to the recognition of (i) an increasing number of patients presenting with a radicular pain syndrome only; (ii) patients with lymphocytic meningitis with a headache lasting months and pronounced general symptoms but no meningeal signs; (iii) a different expression of the disease in children; (iv) a rather frequent CNS involvement either as an acute second stage myelitis or as a third stage encephalomyelitis.

According to Åsbrink *et al.* (1986) 10–15% of European patients with an untreated erythema migrans will develop neuroborreliosis. However, absolute incidences of neuroborreliosis have not been available so far. This is because unequivocal case definitions and a reporting system covering a defined and large population are lacking. Due to the unique sampling conditions we believe that incidences and especially relative incidences of the different manifestations obtained in this study are indicative for this disease in general. We used restrictive diagnostic criteria based on the presence of inflammatory CSF changes and intrathecal specific antibody production, criteria similar to those used for the diagnosis of neurosyphilis. The incidence was between 5.7 and 24.1 cases/million/year including one or two annual cases of chronic encephalomyelitis. These are minimum figures, mainly because neuroborreliosis is probably still underrecognized as reflected by the increasing annual number of patients. Based on previous descriptive reports (Gelbjerg-Hansen, 1945; Dalsgaard-Nielsen and Kierkegaard, 1948) the disease has been endemic in Denmark for decades, but as for other European countries so far we have no evidence to prove a real increase of the disease.

The clinical features of neuroborreliosis usually evolve as a characteristic sequence of events, even respecting certain time-limits (Table 4). Whether neuroborreliosis may develop without a preceding erythema migrans is not known, but due to lack of other symptoms, a hidden location or pallor, the erythema may easily pass unnoticed. Considering the latencies shown in Table 4 it is very unlikely that neuroborreliosis will develop if the patient has been asymptomatic for 4–5 mths even after onset of an untreated erythema migrans. The close association of the seasonal onset of neuroborreliosis (Fig. 3) and the seasonal activity of *Ixodes* ticks indicates that *B. burgdorferi*, unlike *Treponema pallidum*, is less liable to persist as a latent asymptomatic neuroinfection. This concept is further strengthened by the fact that only two out of eight patients with chronic encephalomyelitis did not have a continuous preceding disease beginning with a meningoradiculitis. In neuroborreliosis the latencies until onset of second and third stage signs are significantly shorter than in neurosyphilis.

Insulin-dependent diabetes mellitus may possibly worsen the course of neuroborreliosis, as two out of three patients with this condition suffered from a severe course; one 31-yr-old female developed a severe chronic encephalomyelitis and one 33-yr-old male developed a radiculomyelitis with a severe paraparesis.

The classification of Lyme borreliosis into three stages (Steere *et al.*, 1983; Åsbrink and Hovmark, 1988) is useful because it seems to reflect important pathogenetic aspects; second stage neuroborreliosis is in most patients a self-limiting condition in contrast to the persisting nature of third stage chronic encephalomyelitis. Most patients with neuroborreliosis are easily classified. The term chronic should be restricted to those conditions which are not self-limiting and/or show a persistent inflammatory CSF syndrome beyond 6 mths after onset. This group will mainly consist of patients with chronic encephalomyelitis and those with long-lasting chronic meningitis.

The pattern of sensory and motor signs in patients with second stage neuroborreliosis was, as in previous studies (Sindic *et al.*, 1987; Kristoferitsch, 1989), compatible with multiple nerve root involvement. Minor involvement of the spinal cord was present in 3–4%, probably mainly reflecting a local extension of a more severe radicular inflammation. These patients with more severe peripheral paresis were more liable to incomplete recovery (Table 7). Like other authors (Pachner and Steere, 1985; Sindic *et al.*, 1987; Kristoferisch, 1989) we did not find that acute encephalitis is a characteristic clinical feature of neuroborreliosis. However, significant EEG abnormalities in 31% indicated a frequent subclinical cortical involvement, possibly merely due to an extension of meningeal inflammation.

Regarding the high incidence of Bell's palsy, *B. burgdorferi* seems only responsible for a small number (1–10%) of these (Jonsson *et al.*, 1990). A facial palsy in a child, a facial diplegia or a palsy with concomitant pain and general symptoms and/or onset within the relevant season should lead to the investigation for neuroborreliosis. We could not confirm a previous report of frequent involvement of the eighth nerve (Hanner *et al.*, 1989). The clinical features of chronic encephalomyelitis were dominated by signs of a slowly progressive myelitis and resembled those described by Ackermann *et al.* (1985) and Kohler *et al.* (1988). A few adults developed, after radicular pains, a chronic condition with headache and pronounced general symptoms, especially nausea and weight loss, but no signs of meningism. Based on CSF findings lymphocytic meningitis was diagnosed. The diagnosis in these patients was usually considerably delayed (Case 78). Six out of eight patients with chronic encephalomyelitis passed through such a condition. Thus we speculate as to whether patients with symptoms of chronic meningitis will finally develop chronic encephalomyelitis. Whether host factors or spirochetal virulence factors determine the course of the disease as either self-limiting or chronic is unknown. No HLA-DR tissue type association has been found in second stage neuroborreliosis (Kristoferitsch and Mayr, 1984), and patients with chronic neurological disease have not been studied in this respect.

The present study confirms previous reports (Stiernstedt, 1985; Christen and Hanefeld, 1986; Pfister *et al.*, 1986) that neuroborreliosis in children differs from that in adults. Children have significantly less intense radicular pain (Fig. 4) and more frequently slight meningism, headache, fever or general symptoms. Symptoms in children may be very faint and insidious. Monosymptomatic paresis was almost only seen in children. The bimodal age distribution (Fig. 2) may be due to the different pattern of hospital admission depending on the age-related severity of neuroborreliosis. Radiculitic pain, like herpes zoster neuralgia is most dramatic in middle-aged and elderly patients (Fig. 2) and is often the main reason for hospital admission. Neuroborreliosis in younger adults, however, is probably under-recognized since they have less prominent radicular pain and rarely signs of meningism. Children with paresis and/or meningism, however, are more likely to be admitted to hospital.

The demonstration of inflammatory CSF signs is, as in neurosyphilis, essential for the diagnosis of neuroborreliosis. The cytological features in CSF consist of mainly CD4 positive lymphocytes, monocytes and plasma cells (Martin *et al.*, 1988*b*; Kristoferitsch, 1989). The demonstration of oligoclonal IgG or even *B. burgdorferi* specific oligoclonal IgG may be useful in early and late disease (Martin *et al.*, 1988*a*; Hansen *et al.*, 1990). Considering the pronounced CSF changes, clinical signs of meningitis are remarkably rare. This may explain the frequent delay and resistance to perform a lumbar puncture in these patients. According to Kristoferitsch (1989) there is no association of the level of inflammatory CSF changes and the severity of symptoms and signs. Cerebrospinal fluid can possibly be normal within the very first days. In accordance with the natural course of this neuroinfection (Hörstrup and Ackermann, 1973; Wulff *et al.*, 1983; Kristoferitsch, 1989), the CSF cell count declined spontaneously in patients with a long disease duration (Fig. 5). Thus months after culmination patients may have low or even normal cell counts while still having symptoms. However, these patients will have specific intrathecal antibody production. Every patient with an unexplained lymphocytic pleocytosis in CSF should be investigated for neuroborreliosis.

Apart from the chronic distal axonal polyneuropathy associated with the clinically distinct acrodermatitis chronica athrophicans (ACA) (Kristoferitsch *et al.*, 1988), neuroborreliosis has not been described without inflammatory involvement of the neuraxis in Europe. The ACA associated neuropathy is mild and usually follows the topographical extension of the skin disease. In contrast to this, American authors (Halperin *et al.*, 1990*c*; Logigian *et al.*, 1990) describe a frequent and mild axonal distal neuropathy without CSF changes and without other specific stigmata of Lyme borreliosis in parallel.

The interpretation of tests for the *B. burgdorferi* specific immunoreactivity is problematic especially if the clinical presentation is not characteristic. The major problems of Lyme serology are the slow onset of a specific antibody response and the frequently persisting antibody reactivity after previous exposure to the agent. A negative IgG test in serum may only exclude neuroborreliosis if the disease duration exceeds 3 mths (Hansen *et al.*, 1988; Karlsson, 1990). However, the predictive value of a positive test is low if no IgM and or IgG titre kinetics are recorded. An aetiological association of a neurological disease and *B. burgdorferi* is too often merely based on the presence of an immunoreactivity to *B. burgdorferi*, a finding which, in endemic areas as well, may be a coincidence. The predictive diagnostic value of the presence of specific intrathecal antibody production on the other hand is high; it is often demonstrable before seroconversion (Stiernstedt, 1985; Hansen *et al.*, 1988; Hansen and Lebech, 1991) and is very unlikely a coincidental finding. Local specific antibody production may not always be detectable within the first 2–3 wks (Hansen and Lebech, 1991), but will become prominent within a short time even after therapy, as it did in 11 patients in this study.

We regard the lack of unequivocal specific CSF antibody production in a variety of long-lasting neurological disorders attributed to *B. burgdorferi* (Halperin *et al.*, 1989, 1990*a,b,c*; Pachner *et al.*, 1989; Logigian *et al.*, 1990) as problematic. Intrathecal antibody synthesis is often stated, but the specification thereof, i.e. as a ratio OD-CSF/OD-serum > 1 is insufficient because a ratio > 1 may easily cover up an insignificant result. Measurement of intrathecal specific antibody synthesis is increasingly used as a scientific indication of an etiological association. For this purpose results should be presented more convincingly, either by an expression reflecting a positive OD difference between CSF and serum or by the demonstration of *B. burgdorferi* specific oligoclonal bands in CSF.

The majority of patients were treated with high dose i.v. penicillin G. No treatment failures were experienced, judging by the clinical recovery and normalization of inflammatory CSF signs. This is in agreement with other reports (Stiernstedt, 1985; Kristoferitsch, 1989) and remarkable because the therapeutic role of penicillin especially in the treatment of neuroborreliosis has been questioned (Dattwyler *et al.*, 1988). However, firmly verified treatment failures in unequivocal cases of neuroborreliosis treated with appropriate penicillin doses are reported rarely if ever. Therapeutic trials comparing the efficacy of penicillin and cefalosporins in patients with definite neuroborreliosis have not shown differences (Kristoferitsch, 1989; Pfister *et al.*, 1989). Furthermore, our study indicates that antibiotic therapy of uncomplicated erythema migrans only resulted in treatment failures, if either erythromycin or an insufficient dose of oral penicillin was used.

The final morbidity in our patients was low and comparable with other studies (Stiernstedt, 1985; Sindic *et al.*, 1987; Kristoferitsch, 1989). Significant disabling sequelae were mainly reported in patients with CNS involvement. Even in series of untreated patients, a low morbidity has been reported (Sindic *et al.*, 1987; Kristoferitsch, 1989; Krüger *et al.*, 1989), indicating the generally benign character of this neuroinfection. However, antibiotic therapy is indicated because it shortens the disease duration significantly, prevents further development of peripheral paresis and stops the progression of chronic encephalomyelitis. It seems that antibiotic therapy has no influence on the final outcome of a peripheral paresis, present before therapy was started (Pachner and Steere, 1985; Kristoferitsch, 1989). The prognosis of a facial palsy was comparable to the prognosis of Bell's palsy (Peitersen, 1982).

Despite its known aetiology, the pathogenesis of neuroborreliosis is still obscure. A direct involvement of the spirochete is indicated by the prompt effect of antibiotic therapy, by the presence of *B. burgdorferi* in CSF (Steere *et al.*, 1983; Pfister *et al.*, 1984; Karlsson *et al.*, 1990) and by the topographical association of the site of entry of the agent and the localization of the first neurological signs. Biopsies from peripheral nerves (Vallat *et al.*, 1987; Meier *et al.*, 1989) have shown signs of peri- and epineural vasculitis as well as axonal Wallerian degeneration, which is in agreement with neurophysiological studies (Wulff *et al.*, 1983; Vallat *et al.*, 1987). Deposition of immunecomplexes or spirochetal antigens have never been demonstrated. We confirmed previous observations (Bammer and Schenk, 1965; Kristoferitsch, 1989) of a topographical association of a preceding erythema migrans and the first paresis (Table 3). This finding suggests transneural centripetal migration of the organism and must be considered in parallel with haematogenous dissemination. The erythema migrans by itself demonstrates the ability of *B. burgdorferi* to migrate in tissue. Transneural migration might explain the characteristic latencies from onset of erythema migrans to onset of neurological symptoms. The role of autoimmunity possibly triggered by antigenic mimicry of spirochetal antigens with nerve tissue (Martin *et al.*, 1988*b*; Sigal and Tantum, 1988; Aberer *et al.*, 1989; Baig *et al.*, 1991) is still unclear.

Whereas the European literature reports quite a uniform clinical picture of neuroborreliosis (Stiernstedt, 1985; Sindic *et al.*, 1987; Kristoferitsch, 1989), the spectrum of neurological manifestations which has been attributed to *B. burgdorferi* in America is much broader (Reik *et al.*, 1979; Pachner *et al.*, 1989; Halperin *et al.*, 1989, 1990*a,b,c*; Logigian *et al.*, 1990). The differences could be due to strain differences on the two continents (Wilske *et al.*, 1988). However, they may also reflect different stringency of diagnostic criteria, especially the acceptance of both normal CSF and the lack of intrathecal specific antibody production combined with an undue reliance on the presence of a specific immunoreactivity in blood. Neuroborreliosis has even been called 'the new great imitator', a term which, according to our experience, is misleading.

We conclude that neuroborreliosis is a common, clinically characteristic but still under-recognized neurological syndrome. The examination of CSF and the search for specific intrathecal IgM and IgG antibody production is essential and will usually make it easier to discriminate neuroborreliosis from a number of differential diagnosis: Guillain-Barrè syndrome, herpes zoster radiculitis, lymphocytic meningitis of other aetiologies, meningeal carcinomatosis, CNS lymphoma, sarcoidosis, multiple sclerosis and other encephalopathies.

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WAVELENGTH DISCRIMINATION IN BLINDSIGHT

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SUMMARY

In the circumscribed, long-standing, clinically absolute visual field defects of three patients with vascular lesions that involved the optic radiation and visual cortex, forced-choice discrimination between coloured stimuli was tested. Paired stimuli were matched for luminous efficiency on the basis of previous measurements of increment-threshold spectral sensitivity made in the same patients and at the same retinal positions. To different extents all patients could discriminate between narrowband wavelength stimuli. The results imply that despite the effects of retrograde degeneration on thalamic and retinal colour-processing channels, neurons which process wavelength information are still functional, although the information they transmit is not consciously perceived.

INTRODUCTION

In human and non-human primates, damage to the striate cortex causes retrograde degeneration in the corresponding parts of the dorsal lateral geniculate nucleus (dLGN) and the retinal ganglion cell layer (Van Buren, 1963*a,b*). The transneuronal degeneration in the retina selectively affects the Pβ (primate beta) ganglion cells (Cowey *et al.*, 1989) that project to the parvocellular portion of the dLGN (Leventhal *et al.*, 1981; Perry *et al.*, 1984) and provide the retinal input for the colour-opponent channels (De Valois *et al.*, 1966; Wiesel and Hubel, 1966; De Monasterio and Gouras, 1975; Schiller and Malpeli, 1977). However, even in the retina of monkeys who had undergone unilateral striate cortical ablation as young adults as long as 8 yrs previously, a subpopulation of Pβ cells escapes degeneration (Cowey *et al.*, 1989; Weller and Kaas, 1989), indicating that as yet unidentified secondary pathways with colour-opponent wavelength processing may exist. Physiological evidence for such pathways is scarce, although Felsten *et al.* (1983) recorded from colour-opponent cells in the lateral pulvinar, and we recently labelled a number of Pβ ganglion with horseradish peroxidase (HRP) from the inferior pulvinar (Stoerig *et al.*, 1991). Even though it is impossible to exclude the possibility that these cells were labelled from fibres of passage to other nuclei, it suggests that some Pβ cells have non-geniculate targets.

In addition, behavioural evidence comes from studies of residual visual functions in the homonymous visual field defects produced by the lesions. Information about the presence, location, orientation, direction, movement and wavelength of visual stimuli presented within the defect is processed in monkeys and in human patients, even though the patients claim that they do not perceive the stimuli but are merely guessing. The latter phenomenon prompted the term 'blindsight' (Weiskrantz *et al.*, 1974; *see* Weiskrantz, 1986, 1990; Cowey and Stoerig, 1991, for recent reviews).

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The comparatively few studies of residual wavelength processing in blindsight are especially interesting in view of possible colour-opponent pathways to targets other than the striate cortex. Bender and Krieger (1951) reported different dark adaptation curves for lights of different wavelengths, implying the continued existence of more than one type of retinal receptor and assuming that the adapting lights were matched for luminous efficiency for any single class of receptor. There was also appreciation of colour when stimuli were intense and presented for more than 1 min under dark-adapted conditions. Perenin *et al.* (1980) studied a patient with a complete bilateral cortical blindness who could initially identify large, centrally presented coloured stimuli, although this result could not be repeated in a later testing session. Blythe *et al.* (1987) reported that one of 16 patients consistently identified 635 nm and 530 nm monochromatic flashes as red and green, but they did not use a forced-choice paradigm. Using one, Stoerig (1987) demonstrated red-green discrimination in six out of 10 patients.

The pervasive problem with investigations of wavelength discrimination in blind fields is to eliminate clues from subjective differences in the brightness of the stimuli. The most common solution is to vary spectral intensity so that discrimination based on brightness differences can be distinguished from one based on wavelength. However, variations in spectral intensity could mislead both monkeys and patients into attending to the wrong quality, i.e. brightness rather than colour. This is especially important with patients who, unlike monkeys, receive no immediate feedback about their performance that would enable them to identify which of the two cues is relevant. To solve this problem, we matched luminous efficiency of coloured stimuli on the basis of our previous measurements of the same patients' increment-threshold spectral sensitivity curves (Stoerig and Cowey, 1989*b*, 1991). Those curves, measured on a white adapting background with large, long stimuli to facilitate the colour-opponent system (Sperling and Harwerth, 1971; Snelgar *et al.*, 1987), showed the characteristic discontinuities that indicate a contribution from colour-opponent channels. To see whether the patients would then also be able to discriminate between paired coloured stimuli matched for their luminous efficiency on the basis of these curves, the present experiments were undertaken. The results demonstrate that all three patients were able to discriminate the coloured stimuli at statistically significant levels, albeit to a different extent.

METHODS

Patients

The three patients (P1, P2, P3) have been described previously (e.g. Stoerig and Cowey, 1989*a*, 1991); they have participated in investigations of their residual visual functions over periods ranging from 8 (P1) to 5 (P2) yrs. All three had suffered vascular lesions in the territory of the posterior cerebral artery. As shown by CT- and NMR-scanning, optic radiations and visual cortex were unilaterally affected in all cases. Patient P3's scans, made shortly after the lesion occurred in 1983, show the most extensive damage to extrastriate cortex. Patients P1 and P3, both male, suffered initial memory deficits, which may be related to their fairly anterior extension of their optic radiation damage. Patient P1, who suffered a right-hemispheric lesion, also complained of visual disorientation which initially prevented him from finding his way in his own house and neighbourhood. Patient P2 suffered her first incident in 1977 when she was 20 yrs old. It caused a left-sided homonymous quadrantanopia, which regressed spontaneously, but was reinstated by a second incident in 1984. It has remained stable since. Her scans show a circumscribed hypodense area in the the right occipital lobe, with relatively little involvement of white matter. Clinical details are summarized in Table 1. The patients are highly trained, attentive and cooperative observers.

Visual fields

Visual field perimetry, both dynamic and static, has been regularly repeated over the years during which the patients took part in visual experiments. Whereas both P1 and P2 had very stable defects with reliable and easily reproducible sharp borders, P3's defect has regressed very slowly from a complete right-sided upper field quadrantanopia to an incomplete quadrantanopia with an amblyopic peripheral and a dense central portion (*see* Fig. 1). The field measurements were made under photopic conditions with a Tübinger perimeter (Sloan, 1971). Stimulus size and luminance was occasionally varied (10′ − 116′ diameter; 10 − 1000 cd/m^2). As it is reasonable to use a readily detectable stimulus in order to delineate areas of incomplete functional loss, the data in Fig. 1 were measured with a white 116′, 320 cd/m^2 stimulus on a 10 cd/m^2 white background. This circular stimulus was moved slowly from the periphery towards a central fixation point (30′), and the patients indicated its appearance within their field of vision by pressing a bell button. Fixation was monocular, by covering the eye with the defect in the nasal visual hemifield with an eye-patch. Measurements were repeated along meridians spaced 15° apart. In addition, the stimulus

TABLE 1 PATIENTS

Patient	*Born (yr)*	*Lesion (yr)*	*Field defect*	*Test position (eye/meridian/eccentricity)*
P1	1915	1982	Incomplete hemianopia	OS/225/10
P2	1957	1977, 1984	Quadrantanopia	OS/135/10
P3	1939	1983	Quadrantanopia	OD/60/10

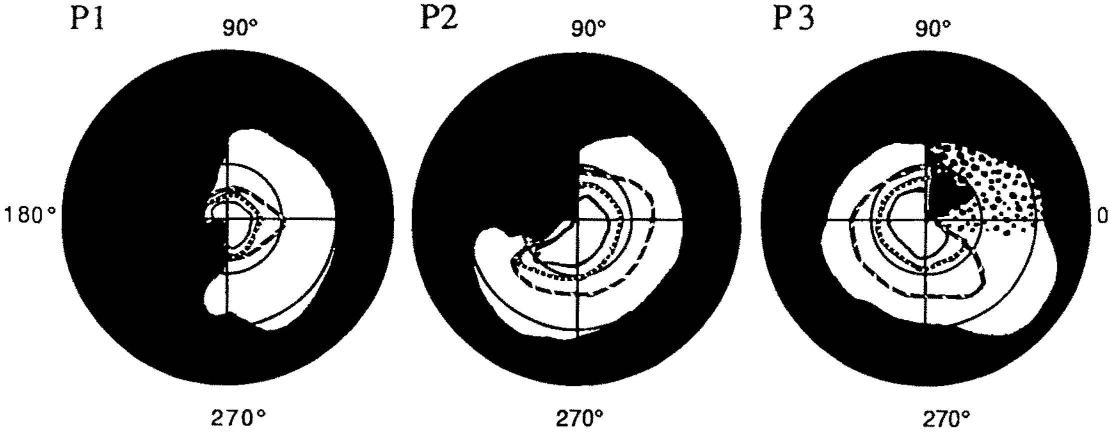

FIG. 1. The visual field plots of the patients' eyes with the defect in the temporal hemifield. A 116′, 320 cd/m^2 circular stimulus was used on a 10 cd/m^2 white background for a combination of static and dynamic perimetry. Areas in which the patients did not detect the stimulus are shown in black, areas of incomplete functional loss are stippled and normal areas are white. The concentric outlines represent colour identification fields measured with blue (dashed, largest), red (dashed, medium) and green (continuous, smallest field) stimuli moved slowly from the periphery towards the fixation point until their colour could be correctly identified.

was moved from inside the field defect to its border with the functional field, and was also presented statically for 200 ms at closely spaced positions in the defective areas to uncover possible areas of incomplete functional loss. Patient P3 could often see the flashed stimulus in the peripheral portion of his defect; however, the perception faded rapidly when a stationary stimulus was presented, or 200 ms stimulus presentations were repeated several times. Areas in which such perceptions occurred are indicated by stippling in Fig. 1, whereas areas in which no response could be evoked are indicated in black.

In addition to the white stimulus, 116′ red, green and blue stimuli were used for dynamic perimetry. They were presented at maximum luminance, i.e 5 cd/m^2 for the blue, 44 cd/m^2 for the green and 20 cd/m^2 for the red. In addition to reporting the first appearance of the stimulus in the peripheral field of vision, the patients verbally indicated its colour as soon as they could identify it. A yellow stimulus was occasionally interspersed as a catch trial. In Fig. 1, it can be seen that the colour identification fields are concentrically organized, with the field mapped with the comparatively dim blue stimulus being larger than red under the present conditions, and red larger than green.

Wavelength discrimination

All tests were performed with the Tübinger perimeter and monocular viewing conditions. The patients fixated a central fixation spot of 30′ diameter with the eye that showed the field defect in the temporal hemifield, which corresponds to the nasal retina. This arrangement was also used in previous experiments (e.g. Stoerig, 1987; Stoerig and Cowey, 1989*a*,*b*, 1991) to ensure that the stimuli fell into a region of the defect whose representation was destroyed for both eyes; the field defect in the temporal hemifield with its numerically greater neuronal representation is generally slightly smaller. Fixation was monitored with a video-system integrated into the perimeter that projected the subject's eye onto a VDU on the experimenter's side of the apparatus. The stimuli (*see below*) appeared for 200 ms on a white background of 10 cd/m^2, which is a low photopic level. Positions were 10° eccentric from the fixation axis (*see* Table 1). Two stimuli were presented in pseudo-random sequence at some particular overall ratio, and the patients' task was to guess which one had appeared. The inter-stimulus interval was approximately 2 s. Each presentation was accompanied by a click emitted from the perimeter shutter that informed the patient that he/she should respond. The response consisted of the colour name, i.e. 'orange' or 'yellow'. Before each block of 250 presentations the patients were informed about which two colours would be used, and could inspect them foveally if they wished. Then, 10 presentations were given in which the experimenter identified the stimulus while the patients fixated the central fixation spot.

Receiver-operating-characteristic curves (ROCs; Green and Swets, 1966) were measured by varying the ratio at which the two stimuli were presented. Five ratios were used, namely 4 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 4, because this number of data points is sufficient to define the ROC (Swets and Pickett, 1982). The patients were informed about the ratio (*see* Stoerig, 1987, for reasons). As the two false [e.g. p('orange'/yellow) and p('yellow'/orange)] and the two correct [e.g. p('orange'/orange) and p('yellow'/yellow)] response categories are symmetric only one pair is used in the figures to depict the discrimination performance. Standard errors were calculated from the binomial distribution, and the statistical significance of the performance represented by each of the five ROC points was estimated by χ^2. The number of ROC points that were significant at the 5% level or better indicates overall discriminability, and takes ROC points sitting on either side of the diagonal that represents chance level into account. As a statistically significant discrimination can occur when the patient consistently gives the wrong colour name, e.g consistently guesses 'green' when the yellow stimulus is presented, putting the corresponding ROC point to the right of the diagonal, the number of significant points is a more sensitive measure than the area under the ROC [p(A)]. The area under the ROC was calculated using the trapezoidal rule. It is clear that even a performance represented in five highly significant ROC points can yield an insignificant p(A) value if the ROC points are distributed to both sides of the diagonal (*see* Fig. 3). As an error estimate for the area under the ROC, the area given by the lower end-points of 1.65 × the larger of each pair of standard errors (corresponding to a one-tailed α of 0.05) was chosen. Performance that is no better than expected by random responding is not significantly different from an area of 0 5.

Each ROC point represents a minimum of 250 presentations. However, those measurements of wavelength discriminability in the blind field that were done in all patients (553 nm vs 600 nm, 553 nm vs 580 nm and 580 nm vs 600 nm) were repeated a second time to see whether the results were repeatable. Together, 500 presentations were given per ratio and condition, and the p(A) values are based on 2500 presentations.

Stimuli

The coloured stimuli were produced by inserting narrowband interference filters (Schott) into the projection beam of the perimeter. Stimuli were circular, 116′ in diameter, 200 ms duration. The three stimuli used with all patients appeared green (peak transmission at 553 nm), yellow (580 nm) and orange (600 nm). The luminance of the green stimulus was 17.6 cd/m^2, that of the yellow 16.4 cd/m^2 and that of the orange 18 cd/m^2. The additional stimuli that were used only in P1 looked bluish green (500 nm, 4.5 cd/m^2), green (528 nm, 8.8 cd/m^2) and red (635 nm, 6.9 cd/m^2). Finally, in an attempt to estimate wavelength discrimination thresholds under the same conditions, two further stimuli were introduced, a yellowish green (560 nm) and a yellowish orange (588 nm). All stimuli were presented on a 10 cd/m^2 white background. Transmission curves of all the filters are shown in Fig. 2.

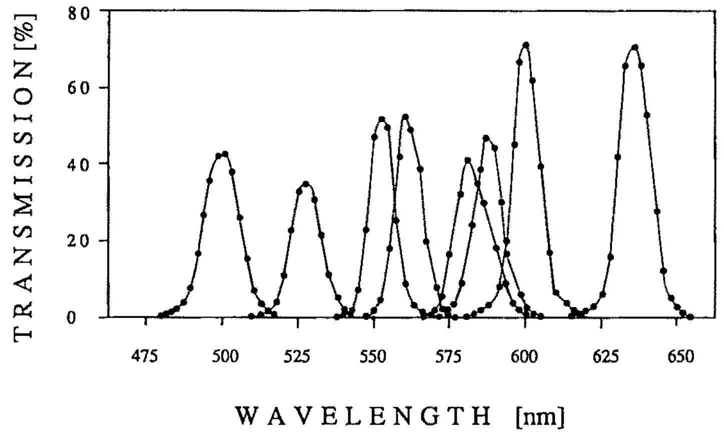

FIG 2. Transmission curves of the interference filters used to test wavelength discrimination.

For the previous experiments on the increment threshold spectral sensitivity of the patients (Stoerig and Cowey, 1991), which were performed under identical conditions at the same retinal positions of the same patients, nine stimulus colours, also produced with Schott narrowband interference filters, were used. Spectral sensitivity was measured by increasing stimulus luminance by 0.1 log units after each block of trials until the patient detected the stimulus, which was given in random order with blank trials, at a statistically significant level. The procedure is described in detail in Stoerig and Cowey (1991). The resultant curves, whose shape was normal for the adaptation level and test conditions, provided the basis for the selection of stimulus luminance. As it was not possible to make large changes in neutral density settings between stimulus presentations, the intensity range of wavelength stimuli was limited. In P2 and P3, the green (553 nm), yellow (580 nm) and orange (600 nm) stimuli could be matched at 0.5 log units above threshold. The choice of stimuli from the middle range of the spectrum allowed us to use interference filters which transmitted light of very similar luminous efficiency for a given neutral density setting, and made a differential contribution from the luminance system, with its broad peak in sensitivity around 555 nm, unlikely (King-Smith and Carden, 1976). For comparability, P1 was tested with the same three stimulus pairs, although only the green and yellow stimuli could be readily matched, at 0.4 log units above threshold. As his sensitivity for the orange stimulus was 0.5 log units higher than that for both green and yellow, and the design of the perimeter made it impossible to introduce such a large change without lengthening the interstimulus interval of 2 s, the 0.5 log unit difference between orange and the two other stimuli was maintained. A possible difference in brightness between the orange and yellow or green stimuli in P1 may ensue, although the variance seen in the spectral sensitivity curves could have been due to fluctuations in sensitivity between different testing sessions. However, the additional green (528 nm) and red (635 nm) stimuli used with this patient were matched at 0.5 log units above threshold and the bluish-green (500 nm) and yellow (553 nm) stimuli at only 0.3 log units above threshold. Stimulus luminance is given above.

Wavelength discrimination thresholds

To estimate the possible increase in wavelength discrimination thresholds in the field defect, two additional stimuli (560 nm and 588 nm) were introduced between the 553 nm green and the 600 nm orange, providing a total of five stimuli in this part of the spectrum. The resulting 10 stimulus pairs were individually tested at the matched 10° eccentric positions in the normal and defective hemifields of P2 and P3. As nasal and temporal hemifields may differ in their wavelength discrimination capacity, the 10° eccentric position in the normal temporal hemifield of the patient's other eye was also tested. The same measurements were made in two normal control subjects, PS and PB. Stimulus luminance was set at 0.5 log units above increment threshold. Thresholds were determined for all positions with an ascending method of limits, using the guessing method described above for the positions in the defects. In the field defects, the threshold measurements were repeated to allow for brightness differences that may result from threshold fluctuations. Positions and luminances are shown in Table 2. Two hundred and fifty presentations were given in the field defect. In the normal hemifield, 50 presentations were given if the subjects could easily tell the colours apart, but 100 presentations were given with the narrowly spaced pairs.

Control measurements

Wavelength discrimination in P3's amblyopic visual field. To see whether the stimuli could be discriminated on the basis of differences in brightness, measurements were repeated at a 30° eccentric position on the 60° meridian in the peripheral portion of P3's defect. At this position, the patient can detect and see the stimulus, but cannot identify its colour. The increment-threshold spectral sensitivity curve for this position had been previously determined (Stoerig and Cowey, 1991), and luminance was again set at 0.5 log units above threshold. Receiver-operating-characteristic curves were measured for the 553 : 600 nm, 553 : 580 nm and 580 : 600 nm stimulus pairs that were tested in all patients.

Colour discrimination in the far periphery of the intact field. In the far periphery, outside of the colour fields determined by dynamic perimetry, colour vision is considerably reduced. However, it is not absent, and enlargement of stimuli in accordance with the cortical magnification factor can compensate for the reduction up to an eccentricity of 80° (van Esch *et al.*, 1984). Even small coloured stimuli, when presented for 200 ms, can often be distinguished because one may appear to have a slightly different tint from the other. The extent of this ability differs between individuals. Whereas P2 could discriminate the 116′ diameter 553 nm, 580 nm and 600 nm stimuli at 40° eccentricity in her intact upper nasal hemifield, P1 reported no sensation of colour when the same stimuli were presented at 40° eccentricity on the 315° meridian, i.e. in his lower nasal hemifield. Patient P1 was therefore asked to try to tell them apart on the basis of whatever he might perceive as different, for example brightness. Stimulus luminance was set at 0.5–0.6 log units above the mean threshold as previously determined at this position; only the luminance of the 528 nm vs 635 nm pair could not be appropriately adjusted for spectral sensitivity at this position (Stoerig and Cowey, 1989*b*, 1991). As for the blind field, ROCs were determined using the same five ratios of stimulus presentation.

Control measurements in the field defect. With P2, control measurements were made with the orange-green pair of coloured stimuli only, because this is the easiest discrimination in that peak transmissivity is about 50 nm apart; it is also the only pair with which she achieved a statistically significant p(A) value in her field defect. To make sure that subjective differences in brightness did not account for this result, we tried to eliminate any colour sensation by presenting the stimuli at 40° eccentricity on the 45° meridian in her good field, but she could still discriminate them by hue. We therefore decided to use stimuli that should be more effective for the luminance system. They were 27′ in diameter and were presented for 200 ms with a flicker frequency of 10 Hz (i.e. two 50 ms flashes per presentation) without the white photopic background, but under photopic conditions at 10° eccentricity on the 45° meridian. Thresholds were measured for nine stimuli from 450 nm to 660 nm. At detection threshold, stimuli from 480 nm to 600 nm appeared yellowish, and the 553 nm and 600 nm stimuli had the same threshold luminance. These two stimuli were then used for the blind field, at the 10° eccentric position on the 135° meridian as in the wavelength discrimination measurements. Their luminance was 44 cd/m^2 for the green and 45 cd/m^2 for the orange stimulus, which was 1.25 log units above the thresholds determined in the good field.

TABLE 2

	Subjects				
Peak wavelength (nm)	*P2 Normal nasal OS/45*	*P2 Normal temp OD/45*	*P2 Blind temp. OS/135*	*P.S. Normal nasal OS/60*	*P S. Normal temp. OS/120*
553	1.74	2.76	21.6	1.95	2.76
560	1.92	2.69	27.65	1.1	2.64
580	1.80	2.54	26.3	1.29	1.96
588	1.49	2.38	21.18	1.92	2.62
600	1.56	2.82	22.2	1.77	2.82
	P3 Normal nasal OD/120	*P3 Normal temp. OS/120*	*P3 Blind temp OD/60*	*S.B. Normal nasal OD/225*	*S B. Normal temp. OD/315*
553	3.0	5.71	27.9	2.44	1.95
560	2.64	6.39	35.35	2.98	2.45
580	2.77	4.62	26.3	2.54	3.18
588	2.08	3.83	28.0	2.38	1.92
600	3.08	4.5	28.8	2.49	2.02

The luminance of the five stimuli used to estimate wavelength discrimination thresholds in P2 and P3 and in two control subjects, P.S. and S.B., is given in cd/m^2. Luminance was set 0.5 log units above detection threshold as determined in the same testing session, and stimuli were presented at 10° eccentricity on a 10 cd/m^2 white background. For each subject the eye (OS, left; OD, right) and the meridian are given.

Luminance-difference thresholds in the good field. At 10° eccentricity in the normal nasal hemifield, on the meridian that with respect to the vertical meridian was mirror symmetric to the one used for testing colour discrimination in the blind field, luminance difference thresholds were measured to determine the just detectable difference in intensity. A coloured stimulus of maximal luminance (116′, 200 ms, as in the experiments on colour discrimination) was paired with a second stimulus which was identical apart from a 0.1 log unit difference in intensity. Bright and dark stimuli were presented in pseudo-random sequence with a ratio of 1 to 4 because this seems to be an especially sensitive condition (*see* Figs 3–5). The task consisted in guessing which of the two was darker, and the luminance difference was increased in 0.05 log unit steps until performance was statistically significant. Measurements were repeated with all stimulus colours used for wavelength discrimination tests. Guessing was used in order to make the measurements and criteria for discriminability as similar as possible to those used in the blind field.

The same measurements were made in one of the authors (A.C.) to see whether luminance-difference thresholds in the patients' normal hemifields were within a normal range, and whether thresholds differ between normal nasal and temporal hemifields. The 10° eccentric positions on the 225° and 315° meridians were tested. As in the patients, thresholds were determined by guessing.

Luminance-difference thresholds in the field defect. To estimate the minimum luminance difference for statistically significant discriminability in the field defect, stimulus pairs of the same colour, but different luminance, were presented at the retinal positions used for wavelength discrimination tests. The brighter stimulus had maximum possible luminance and was presented for 200 ms in random sequence with a 0.5 log unit darker stimulus. The patients guessed whether the just presented stimulus was dark or bright. A ratio of one bright to four dark stimuli was used, and 250 presentations given. If performance was significant, the luminance difference was increased by 0.1 log units and measurements were repeated. If performance was not significant, the luminance difference was increased accordingly.

Perception (or lack thereof)

Throughout the sessions, each of which lasted for 2–3 h over a period of several months, the patients were repeatedly asked whether they had any visual or other experience in relation to stimulus presentation in the field defect. They consistently, repeatedly and firmly said that they did not experience anything.

RESULTS

Wavelength discrimination

Orange vs green

With the green and orange stimuli, whose peak transmission was 50 nm apart, all three patients showed statistically significant discrimination. The number of ROC points significant at the 5% level or better is five for P1, three for P2 and three for P3. These curves are shown in the upper row in Fig. 3, with significance indicated by asterisks. One asterisk indicates significance at the 0.05 level, two indicate significance at the 0.005 level or better. The measurements were repeated in later test sessions, and the results were very similar, as shown in the lower row of curves in Fig. 3. Patient P1 produced four significant ROC points, and P2 and P3 both scored three, as before.

Yellow vs green

When the green (553 nm) and yellow (580 nm) stimuli were paired, P1 and P3 again performed statistically significantly in both first and second test series. In contrast, P2 showed just one (out of 10) significant ROC point at the 0.05% level. Again, the curves were very similar for both series and in Fig. 4 the combined data are given, each curve representing 2500 presentations (instead of 1250, as in Fig. 3).

Orange vs yellow

Discrimination of the orange (600 nm) and yellow (580 nm) stimuli, whose peak transmission is just 20 nm apart, is again statistically significant for P1 for nine of the 10 ROC points. Patient P3's curves contain five significant points, and P2's contain

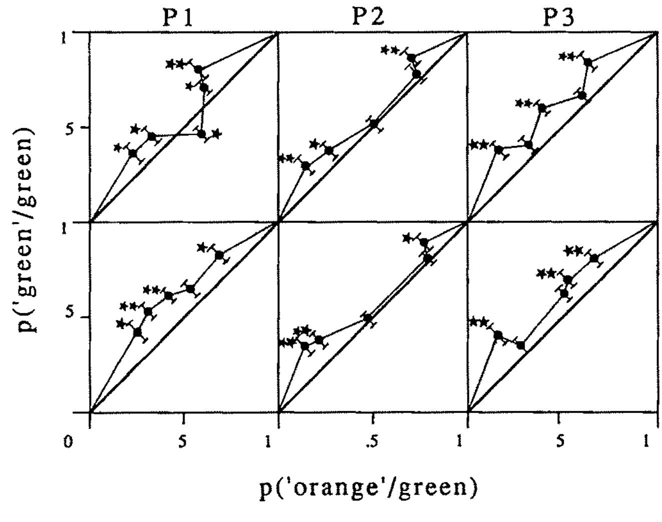

FIG. 3. The receiver-operating-characteristic curves (ROCs) represent discriminability of orange (600 nm) and green (553 nm) stimuli at 10° eccentric positions in the field defects. Each curve is based on 1250 stimulus presentations. Results for first (*upper row*) and second test series are very similar. Statistically significant ROC points ($*P < 0.05$; $**P < 0.005$) are numerous. All patients' ROCs give evidence of orange-green discrimination.

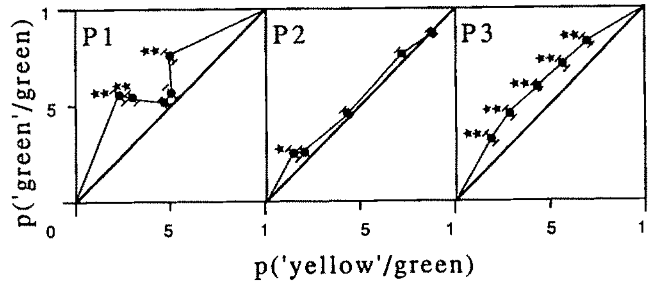

FIG. 4. Results from the two series of yellow-green (580 nm vs 553 nm) discrimination are added so that each ROC represents the result of 2500 measurements Receiver-operating-characteristic curve points are given with the larger of each pair of standard errors drawn orthogonal to the diagonal that indicates chance performance Whereas P1 and P3 perform well above chance, P2's performance is at chance level ($*P < 0.05$; $**P < 0.005$)

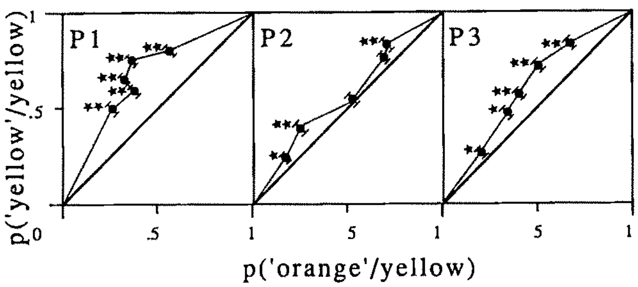

FIG 5 Receiver-operating-characteristic curves representing yellow (580 nm) and orange (600 nm) discrimination are again based on 2500 measurements each. Patients 1 and 3 perform as well with orange as with green, but P2's ROC is not significantly different from the diagonal that indicates chance performance, despite the presence of individually significant points ($P < 0.05$; $**P < 0.005$).

three. Again, data from both test series are very similar, and are combined in Fig. 5. The results of the measurements are summarized in the histograms in Fig. 6. The values of p(A), as single indices of discriminability and calculated from 2500 presentations, are given in the left-hand histogram. They show that P1 performed equally well with all stimulus pairs, even though only the green-yellow pair was matched at 0.4 log units above his detection threshold. Patient P2's performance deteriorated with decreasing separation in peak transmissivity; with the yellow-green and orange-yellow pairs, the area under the curve is not significantly different from 0.5. Patient P3's discrimination, like P1's, was almost equally good with all stimulus pairs, although the p(A) value is slightly lower for the orange-yellow discrimination, indicating that the wavelength discrimination was close to threshold (*see below*).

The number of significant (5% or better) ROC points is given in the histograms to the left in Fig. 6. It can be seen that P2 scores three significant points with the yellow-orange pair, although the corresponding p(A) value is not significant. Indeed, the patients' overall performance is best with this pair; collectively, they have 13 signi-

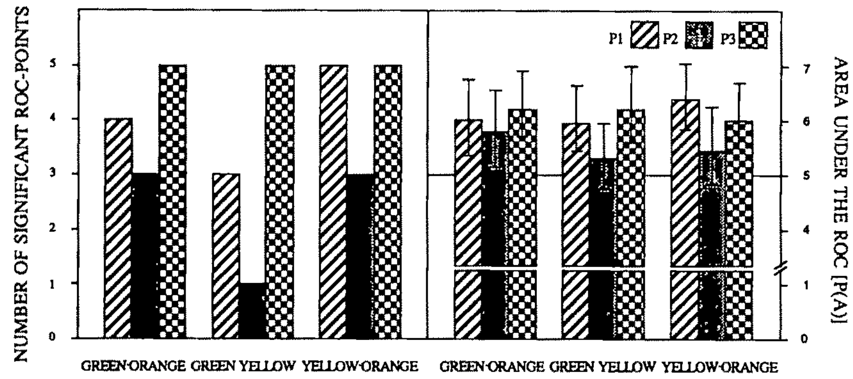

FIG. 6. The left histogram gives the number of ROC points that are significant on the 5% level or better The right histogram gives the area under the ROC as single index of discriminability The error estimate gives the area given by the lower end-points of 1 65 × the larger of each pair of standard errors drawn orthogonal to the diagonal. Overall performance is significant for P1 and P3 in all three discriminations, but in the orange-green discrimination only in the case of P2.

ficant points, as compared with 12 for the green-orange and nine for the green-yellow discrimination.

Additional measurements in P1

Patient P1 was tested with two additional stimulus pairs. Receiver-operating-characteristic curves based on 1250 presentations were determined. In the red-green (635 nm vs 528 nm) discrimination, with stimuli matched at 0.5 log units above threshold, the patient scored four significant ROC points, and had a p(A) value of 0.61 ± 0.09. This is very similar to the yellow-green (580 nm vs 553 nm) discrimination, and shows that an increase in wavelength separation does not substantially improve discriminability. The yellow (580 nm) and bluish-green (500 nm) stimuli were matched at only 0.3 log units above threshold. He still scored three significant ROC points, and again had a p(A) value of 0.61 ± 0.09, showing that even with stimuli that are closer to detection threshold discrimination remained statistically significant.

Wavelength discrimination thresholds

Ten stimulus pairs, with peak transmission from 553 nm to 600 nm, were used in an attempt to estimate the wavelength discrimination in normal and blind hemifields. The separation of peak filter transmission, in nanometres, was 47 (553 : 600), 40 (560 : 600), 35 (553 : 558), 27 (553 : 580), 28 (560 : 588), 20 (580 : 600 and 560 : 580), 12 (588 : 600), 8 (580 : 588) and 7 (553 : 560). The patients were tested at three 10° eccentric positions, namely in their normal nasal, normal temporal and blind temporal fields. In her normal field, P2 could discriminate the wavelengths perfectly down to a separation of 20 nm, and was above chance with all but the 553 : 560 nm pair. In the field defect, her performance was significant only with two of the most separated pairs (553 : 600 nm and 553 : 588 nm). In his normal field, P3 could significantly discriminate all pairs, with the exception of the last pair, which he could not tell apart in his normal nasal hemifield. In his defect, he performed above chance with all pairs down to a separation of 20 nm (*see* Fig. 7). Like P3, the control subjects could discriminate between all stimuli. They showed perfect discrimination for the first five or six pairs, which incidentally are the same pairs that P3 could discriminate in his field defect, and still significant, but reduced discriminability for the closer spaced stimuli. No difference was observed between the normal fields of patients and controls.

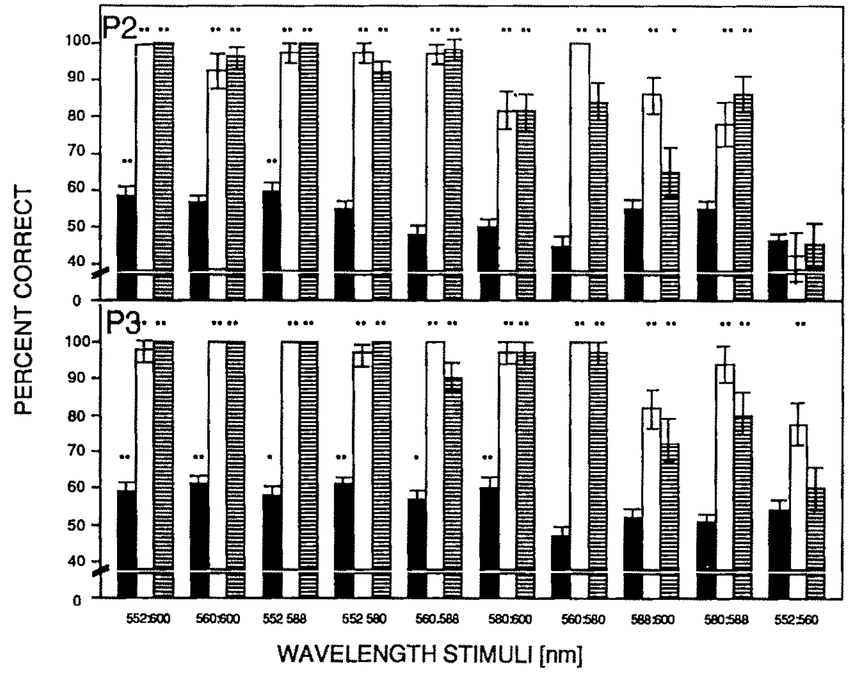

FIG. 7. Wavelength discrimination measured with 10 stimulus pairs at an eccentricity of 10° in the blind temporal field (filled bars), normal temporal field (empty bars) and normal nasal field (striped bars) of P2 (*top*) and P3 (*bottom*). In the field defect, discrimination is significant down to 35 nm and 20 nm wavelength separation, respectively. Although discrimination in the normal fields becomes more difficult from 18 nm separation onwards, guessing maintains it at a significant level down to the 7 nm separation (553 : 560 nm). With this pair P2 fails to reach a significant performance in both normal hemifields, and P3 in the nasal one, as indicated by asterisks ($*P \leq 0.05$, $**P \leq 0.005$).

Control measurements

Wavelength discrimination in the amblyopic field of P3

To test whether our match for luminous efficiency was effective we used the peripheral amblyopic portion of P3's field defect. For two reasons, this portion of the visual field is particularly suited for such a control experiment. First, small targets provide sensations only of brightness, not colour, so that any discriminability must be based on luminance differences. Secondly, spectral sensitivity at the 30° eccentric position used for these measurements is fairly variable in this subject (Stoerig and Cowey, 1991) so that brightness differences might still exist when stimuli are matched on the basis of the mean of data gathered in different testing sessions.

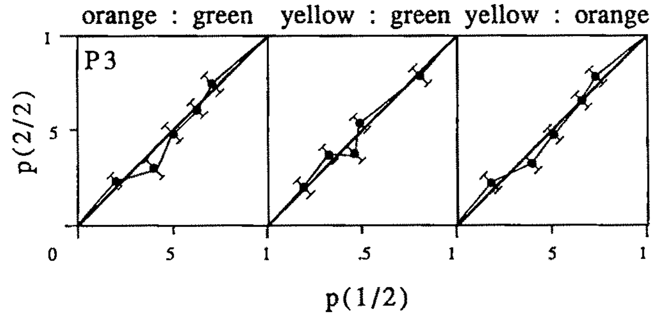

FIG 8. No evidence for statistically significant discrimination is seen when a 30° eccentric position in P3's amblyopic field is tested with stimuli matched for luminous efficiency on the basis of previous measurements of spectral sensitivity made at the same position.

As at the 10° eccentric position in his blind field, the three stimuli (600 nm, 580 nm, 553 nm) were presented in pairs. However, a 30° eccentric position on the same 60° meridian was now used. The patient reported seeing the stimuli; they appeared colourless, occasionally fading and disappearing altogether and then returning again during a series. He was instructed to try to tell them apart by brightness or whatever other difference he might perceive, and to guess, as with earlier measurements in the blind field, when he could not see a difference. Note that the luminance of the stimuli was chosen in accordance with increment-threshold measurements made at the same 30° eccentric position now used for the control tests.

The results are given in Fig. 8 where each curve represents 1250 stimulus presentations. No ROC point is significant, nor do the p(A) values differ significantly from 0.5. For the easiest discrimination (green vs orange) the measurements were repeated as in the blind field to yield a ROC that represents 2500 trials. The curve that represents both series of measurements still has no significant ROC point; the p(A) value, 0.498 ± 0.068, is not significantly different from 0.5. In contrast, when other stimuli with their luminance matched for the spectral sensitivity measured at 10° eccentricity in the blind field were used instead, discriminability was highly significant, showing that the patient could discriminate on the basis of brightness differences.

Wavelength discrimination in the far periphery of the normal hemifield of P1

Control measurements were also made at a 40° eccentric position in the normal hemifield of P1. Again, increment-threshold spectral sensitivity had previously been determined under identical conditions (Stoerig and Cowey, 1991), and stimulus luminance was set at 0.5–0.6 log units above threshold. However, compared with the amblyopic visual field, the far periphery (40°) of the normal hemifields is less well suited for these control experiments because although the test positions lay outside of the colour fields established with dynamic colour perimetry, static perimetry often reveals discrimination of stimuli of different colour, which is based on hue rather than brightness differences. Nevertheless, P1 said he had to guess which stimulus was which, and that he could not see a difference that would allow him to discriminate the two targets when

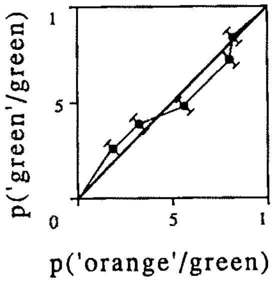

FIG. 9. Patient P2 could not discriminate green (553 nm) and orange (600 nm) stimuli at 10° eccentricity in her field defect when the stimuli were 27′ in diameter, flickered at 10 Hz, and were presented without the adapting white background to favour the luminance system that is especially sensitive to brightness differences.

they were presented at 40° eccentricity on the 315° meridian. No ROC point was significant. The p(A) values were 0.542 ± 0.105 for green vs orange, 0.464 ± 0.08 for green vs yellow and 0.483 ± 0.10 for orange vs yellow, none of which is significantly different from 0.5.

Wavelength discrimination in the field defect of P2 under conditions favouring the luminance system

Patient P2 could tell the orange from the green stimulus at 40° eccentricity on the 45° meridian in her normal hemifield. Therefore, conditions were altered for the control measurements in order to favour the luminance instead of the colour-opponent system: under photopic adaptation, 27′ stimuli flickering at 10 Hz were presented for 200 ms without the white adapting background. Their luminance was set at 1.25 log units above the threshold values determined for these stimuli at 10° eccentricity on the 45° meridian in the patient's good upper hemifield, and corresponds to a luminance 0.4 log units higher than that of the stimuli used for wavelength discrimination. Figure 9 shows that the alteration of experimental conditions eliminated all evidence of discriminability even though the luminance system should be better suited to detect brightness differences.

Luminance discrimination thresholds

(i) *Normal hemifield.* In Fig. 10, the luminance discrimination threshold values for the 10° eccentric positions in the normal hemifield of the patients are indicated with open symbols. For better comparability with the data for the blind field, they were determined by asking the patients to guess whether the brighter or the darker stimulus had appeared. The brighter 116′ stimulus always had maximum available luminance, and was presented once for every four presentations of the darker companion. This uneven ratio was also used in the field defect because it often yields better detectability or discriminability than one in which the two targets have equal probability of appearing (*see* Figs 3–5). Tests began with a 0.1 log unit luminance difference which was increased in steps of 0.05 log units after each series of 100 presentations, until performance became statistically significant. The value at which performance reached the 5% level of significance was defined as luminance difference threshold. These threshold values are in the order of 0.1–0.3 log units, whereas those derived from judging instead of guessing ranged between 0.15 and 0.6 log units for a reliable sequential same-darker judgement. There is no clear dependence of this threshold on the stimulus wavelength.

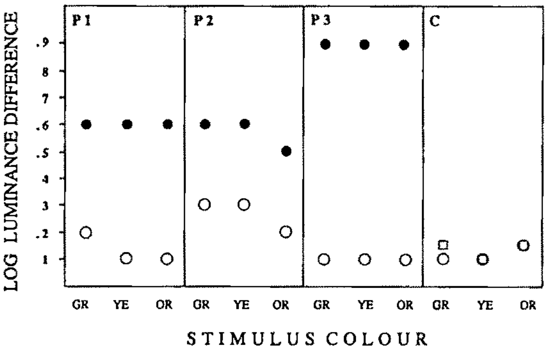

FIG 10. Luminance difference thresholds were measured with brighter and darker targets of the same wavelength at 10° eccentricity in the normal nasal (○) and blind temporal (●) fields. Stimuli were presented sequentially, and the patients guessed which stimulus was brighter, which darker. The luminance difference at which the performance became statistically significant was defined as threshold. Data for the control subject, also determined by guessing, show no difference in sensitivity between normal nasal (○) and normal temporal (□) hemifields.

Measurements were also made on A.C., a normal trichromat who had no history of ophthalmological or neurological disease, to see whether the patients' thresholds in the normal hemifield were within a normal range, and to estimate the difference at positions of identical eccentricity in nasal and temporal hemifields. When the judging method and a 100% correct criterion for discriminability were used, the luminance difference had to be between 0.3 and 0.45 log units in the temporal hemifield (10° eccentricity, 225° meridian) and between 0.3 and 0.55 at the corresponding position in the nasal hemifield (315° meridian). These values are in the same range as the patients, and show that the temporal hemifield, corresponding to the nasal retina, may be slightly more sensitive. With the guessing method, the luminance difference necessary for statistically significant discrimination dropped to 0.1–0.15 log units and no nasotemporal difference was observed.

(ii) *Field defect.* In the field defect the brighter stimulus, again of maximum luminance, was given in random sequence with the darker one at a ratio of 1 : 4. In a first series the difference was 0.5 log units. Two hundred and fifty presentations were given, and the patients guessed whether the darker or the brigher stimulus had been shown. In cases where the result was statistically significant with this luminance difference, it was reduced by 0.1 log units, and this was repeated until performance declined to chance level. When the percentage correct value of luminance difference of 0.5 log units was not significant, the difference was increased in steps of 0.1 log units until it became significant. The resultant values which are shown in filled symbols in Fig. 10 range from 0.5 to 0.9 log units. It is interesting that P3, who showed good discriminability even of orange and yellow stimuli, performed comparatively poorly at this task; in fact, he required a luminance difference so large that the darker stimulus approximated his previously determined detection threshold, implying detection rather than discrimination.

The difference between the difference thresholds in the normal nasal and blind temporal hemifield of the same eye is between 0.3 and 0.8 log units. Considering that the temporal hemifield normally has the same or slightly better sensitivity, the decrease in sensitivity, or increase in threshold, is approximately 0.3–0.9 log units in the blind temporal hemifield. To be discriminable on the basis of brightness differences, the coloured stimuli used to test wavelength discrimination would therefore have to differ in intensity by at least that much.

DISCUSSION

When wavelength discrimination was tested with stimuli matched for luminous efficiency on the basis of spectral sensitivity curves determined at the same 10° eccentric position within the field defects, all three patients showed clear evidence of being able to discriminate between two or more stimuli at statistically significant levels. Although the p(A) values range between 0.58 and 0.62 only, it should be remembered that the stimuli were deliberately made close to the detection threshold in order to remain isoluminant, and to minimize stray light effects. Patients P1 and P3 performed as well, or almost as well, with green vs yellow and orange vs yellow as with green vs orange stimuli; a larger separation in peak transmissivity, as in the additional red-green (635 nm vs 528 nm) discrimination in P1, did not improve discriminability. In the bluish-green/yellow (500 nm vs 580 nm) discrimination, P1 still performed in a statistically significant manner, even though the stimuli were matched at only 0.3 log units above his previously determined detection threshold, demonstrating that discriminability is upheld even in the near-threshold range. For P1, the orange stimulus could not be matched for luminance with the green (553 nm) and yellow (580 nm) ones. Despite the mismatch the results of all discriminations are fairly similar in this patient, and in particular show no differential improvement with the two mismatched pairs (553 nm vs 600 nm and 580 nm vs 600 nm). In view of the normal shape of the spectral sensitivity curve, it is likely that the difference in sensitivity, with the threshold for the orange stimulus being lower than those for both the green and yellow stimuli, arose as a consequence of the extended testing procedures which make it necessary to determine the different thresholds in different sessions. As the mismatch is in the order of 0.5 log units, with the green and yellow stimuli at 0.4 and the orange at 0.9 log units above threshold, and the patient's separately determined luminance difference threshold was 0.6 log units (Fig. 10), it is unlikely that his discrimination was based entirely on a brightness difference. However, the luminance difference may have enhanced discrimination, especially for the yellow-orange pair where he scored five significant ROC points as compared with the three in the yellow-green (580 nm vs 553 nm) discrimination.

Compared with the other two patients, P2 showed a poorer performance with the closer wavelengths. Her performance was significant only with the two pairs of well-separated stimuli, requiring a minimum separation of 35 nm in the middle part of the spectrum. As the 560 : 600 nm pair was tested in the equal probability condition only, we recognize that she might have shown evidence of discrimination for this pair as well as with more extended testing, particularly as the 1 : 1 stimulus ratio is a difficult condition. Finally, P3 was able to discriminate all pairs down to 580 : 600, i.e. 20 nm separation, at statistically significant levels.

The results therefore demonstrate that information about wavelength can be transmitted from within field defects, and that there are individual differences in discrimination ability. Patients P1 and P3 could discriminate stimuli as close together as 20 nm, whereas P2 required 35 nm separation. Such differences are also seen in normal observers, and in the normal hemifields of the patients. With our limited set of interference filters, we cannot give exact discrimination thresholds for the normal hemifield. However, threshold was reached in both normal hemifields of P2 and in the nasal hemifield of

P3 at 7 nm separation, and forced choice guessing was necessary to discriminate even the 12 nm separated pair with statistical significance. This is in accordance with estimates from other studies of wavelength discrimination in the periphery of the normal visual field, which show that thresholds are considerably higher at an eccentricity of about 10° than at the fovea (*see* Moreland, 1972, for review), even when the eccentric stimulus is larger in order to scale it for magnification factor (van Esch *et al.*, 1984). For the field defect it follows that the increase in discrimination thresholds is three- to five-fold.

Control measurements were performed to check that successful discrimination was based on wavelength and not subjective brightness differences (or on whatever it is in 'blindsight' that corresponds to brightness in real vision). Measurements at 30° or 40° eccentric positions in seeing (normal and amblyopic) regions of the visual field, with the same stimulus colours used in the blind field more centrally but with the luminance settings adjusted to the spectral sensitivity curves measured at these more eccentric control positions, showed that discrimination was impossible when no colour difference was perceived, demonstrating that our matching for luminous efficiency was effective. Interestingly, when the conditions were slightly changed in a control experiment designed to enhance the luminance system (King-Smith and Carden, 1976) and performed at 10° eccentricity in the field defect of P2, performance was no better than expected by chance. As the luminance system is most sensitive to brightness differences, this too demonstrates that they were effectively eliminated with the method of matching for luminous efficiency. In addition, the luminance discrimination thresholds measured under identical conditions in the blind field demonstrated that a minimum difference of 0.3 to 0.9 log units would be required for the patients to be able to discriminate stimuli of the same wavelength on the basis of this feature. Together, these results rule out the possibility that discrimination was based on 'brightness' differences; only the yellow-orange discrimination in P1 remains a possible exception.

Our estimates of luminance difference thresholds are in reasonably good agreement with those measured by Leporé *et al.* (1976) in monkeys with bilateral striate cortical ablation. Using different experimental conditions that included a stimulus of 3.5 × 10° and prolonged prior dark-adaptation even for photopic measurements, they found an increase in differential achromatic thresholds that was approximately threefold, i.e. about 0.5 log units as compared with our 0.4–0.9.

Functional neuroanatomy

How is wavelength processing achieved in a field defect? Three visual subsystems can be distinguished on the basis of their retinal ganglion cell input in the normal brain. The first receives its input from the Pα ganglion cells whose functional characteristics include high contrast sensitivity, high temporal resolution, low spatial resolution and broadband spectral sensitivity. This system is thought to play a crucial role in motion processing (Livingstone and Hubel, 1988; Schiller *et al.*, 1990) and in certain aspects of disparity processing (Livingstone and Hubel, 1988), and is sometimes equated with the luminance system, which gets little if any input from the short wavelength cones (De Valois *et al.*, 1966; Gouras, 1968; Derrington *et al.*, 1984; Lee and Stromeyer, 1989). The second subsystem receives its retinal input from the Pβ ganglion cells. The cells have low temporal resolution and comparatively low contrast sensitivity, but high spatial resolution and prominent colour-opponent properties. This system is associated with the processing of spatial detail and of wavelength. The third subsystem, which receives its input from the Pγ retinal ganglion cells, has been much less extensively studied; it appears to be functionally heterogeneous, comprising cells with unusual centre-surround receptive field organization and motion sensitivity (De Monasterio, 1978). It is associated with saccadic localization (Schiller and Koerner, 1971; Mohler and Wurtz, 1977) and visual attention (Zihl and von Cramon, 1979).

These three subsystems are the candidates for wavelength processing in field defects. Among them, the Pα cell system is probably the least likely. Although the surround region of cells in the magnocellular portion of the dLGN, unlike the centre, is often particularly sensitive to long wavelengths, and magnocellular dLGN cells are never totally insensitive to isoluminant chromatic borders or whole-field changes in isoluminant hues (Hicks *et al.*, 1983; Derrington *et al.*, 1984; Hubel and Livingstone, 1990), this system is not usually associated with wavelength processing. In addition, the lack of wavelength discriminability in the amblyopic field of P3, where vision is transient in its properties, and in the field defect of P2 when experimental conditions favour the luminance system, as well as the marked increase in luminance difference thresholds, show that the Pα system is a poor candidate. Poor wavelength and good luminance discrimination should be its hallmarks.

The Pβ cell system is the most obvious candidate for wavelength processing. The Pβ cells and their target cells in the parvocellular portion of the dLGN are the only cells known to possess colour-opponent properties which enable them to signal wavelength unambiguously. However, the transneuronal retrograde degeneration of the retinal ganglion cell layer (Van Buren, 1963*a*,*b*; Cowey, 1974) is selective for Pβ cells; it progressively destroys up to about 80% (Cowey *et al.*, 1989), depending on the retinal eccentricity, the age at which the lesion occurred (Weller and Kaas, 1989) and poorly understood individual differences (Niida *et al.*, 1990). Although this loss is massive and selective for this subsystem, a portion of these cells survives even 8 yrs after unilateral striate cortical ablation, suggesting that the survivors retain functional connections. As the only known projection area of Pβ cells is the parvocellular division of the dLGN which degenerates almost totally, there are only two possible explanations: either the surviving Pβ ganglion cells are sustained by remaining geniculate neurons, or they project elsewhere in the first place. As regards the first possibility, the surviving geniculate projection neurons that send their axons to extrastriate cortical areas (Cowey and Stoerig, 1989) are far too dispersed for more than a fraction of the surviving Pβ cell axons to innervate them, especially in view of the small terminal arbors of Pβ axons (Michael, 1988). However, the relatively abundant and extensively interconnected interneurons, presumably involved in the provision of spatial and chromatic inhibitory inputs to the projection neurons, may receive input from the surviving Pβ ganglion cells and thereby preserve them. With respect to the second possibility, one possible non-geniculate target is the pulvinar. In either case, the survival of a subgroup of Pβ cells indicates that it is conceivable that wavelength discrimination is mediated by the residues of this system.

The third and last possibility involves the Pγ cells which, like the Pα cells, show no sign of transneuronal degeneration and whose major projection zone, the superior colliculus, remains functional in the absence of striate cortex. Colour-opponency has not been reported in this system (Marrocco and Li, 1977), but many visual neurons in the superior colliculus show band-pass characteristics (Kadoya *et al.*, 1971), responding to short, medium or long wavelengths. This alone is insufficient to signal wavelength independently of luminous intensity. But if the outputs from cells in this region to the inferior pulvinar (Benevento and Fallon, 1975; Harting *et al.*, 1980) and to the surviving projection neurons in an otherwise degenerated dLGN (Kisvárday *et al.*, 1991) were combined via interneurons in an opponent fashion, genuine wavelength discrimination, based originally on the Pγ cell projection to the superior colliculus, could be possible. However, at present it is not even clear whether the wavelength tuning of the collicular units arise from retinal or from cortical input. Irrespective of which of the three main subsystems is involved, any necessary contribution to wavelength discrimination of their further direct or indirect projection to extrastriate cortical visual areas can be tested with hemispherectomized patients.

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EYE MOVEMENTS IN MYOTONIC DYSTROPHY

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SUMMARY

Smooth pursuit eye movements were studied with infrared reflection equipment in 10 patients with myotonic dystrophy and in 10 age- and sex-matched controls. Smooth pursuit gain, measured after correction for catch-up saccades, was decreased in the patient group. Normal latencies of saccadic eye movements made a lack of attention an unlikely explanation for this low gain. Likewise, presence of catch-up saccades and normal fixation made it unlikely that extra-ocular myopathy explained the low smooth pursuit gain. We suggest that periventricular white matter abnormalities represent a more likely explanation.

INTRODUCTION

Myotonic dystrophy (MD) is characterized by an autosomal dominant pattern of inheritance, muscular atrophy, myotonia and the occurrence of changes in non-muscular tissues such as the lens of the eye, testis and other endocrine glands, the skin and the brain. Oculomotor symptoms such as diplopia are rare in patients with MD, but ptosis is not uncommon. Evidence of central nervous system involvement in MD has been provided (Harper, 1989). Recently, periventricular white matter abnormalities have been described with magnetic resonance imaging (MRI) (Glantz *et al.*, 1988; Huber *et al.*, 1989). Pathways in the periventricular white matter are involved in control of smooth pursuit eye movements in monkeys (Tusa, 1988) and probably also in humans (Thurston, 1988). In a recent electro-oculographic study, gain of smooth pursuit eye movements was found to be normal in patients with MD. Nevertheless, smooth pursuit was interrupted by catch-up saccades (Ter Bruggen *et al.*, 1990), as has also been reported by others (Oohira *et al.*, 1985). This is surprising, since catch-up saccades are the hallmark of defective smooth pursuit. Because of this discrepancy, we examined smooth pursuit in 10 MD patients with an infrared reflection (IR) technique (Reulen *et al.*, 1988) and newly developed software for smooth pursuit analysis, correcting for the confounding influence of catch-up saccades.

Smooth pursuit gain is lowered by somnolence (Leigh and Zee, 1991) which frequently occurs in MD (Harper, 1989). Latencies of saccadic eye movements are known to be abnormally prolonged in somnolent or inattentive subjects (Leigh and Zee, 1991). As a measure of vigilance of our patients, we measured latencies of saccadic eye movements.

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MATERIALS AND METHODS

Horizontal eye movements were measured with an IR technique combined with a computerized eye movement processing system (Reulen *et al.*, 1986, 1988; Reulen, 1987). The eye movement measuring device was incorporated in a spectacle frame attached to the head. Accordingly, eye position was measured with respect to head position. The resolution of the IR technique is 2 minutes of arc. The IR technique allows d.c. recording of eye movement such that after calibration the true eye position is given at any moment. The system is linear within 3% for horizontal eye rotation up to 20°, measured from the central position. The movement of both eyes was measured simultaneously while patients were sitting in a room with a low level of ambient illumination. One of the investigators gently stabilized the head with his hands. All eye movement data were low-pass filtered (100 Hz, −6 dB) and digitized at a rate of 200 samples per second. After digitization, a velocity signal was derived from the position signal by a two point central difference algorithm.

The target for saccades consisted of an array of red gallium-arsenide light-emitting diodes (LEDs) (wavelength 635 nm), mounted in a cylindrical screen with the centre between the eyes of the patient. Each diode subtended a visual angle of 0.16° at a viewing distance of 1.5 m. If viewed from the distance of 1.5 m, the peripheral target spots were located 20.1° to the left and right of the primary fixation position at the centre of the screen. The LEDs were controlled by computer (Reulen *et al.*, 1988). The maximum target luminance was 3 log units above the foveal threshold level. To study visually evoked saccades, target steps of 20.1° randomly selected to the left or right of the primary central fixation position were generated by extinguishing the central LED and simultaneously illuminating a peripheral one. Since the linear operating range of the IR device is 20°, we analysed saccades with amplitudes up to 20°. The time between two successive target jumps varied randomly between 2 s and 4 s, so that the patient could not predict the exact moment of occurrence of the peripheral stimulus. Patients were instructed to change fixation from the central target to the peripheral spot as quickly and accurately as possible. The peripheral target was extinguished after 2 s and the central target was relit simultaneously, evoking a saccade back to the primary position of gaze. As return saccades were predictable, they were not included in the study. Between saccades, the central LED remained lit and was fixated by the patient. Twenty rightward and leftward saccades of every patient and control were studied. Patients were kept alert by verbal encouragement. After each 10th stimulus a short break was introduced to avoid fatigue.

Fixation was tested by asking the patients to fixate on the target presented for 30 s first in the centre of the screen, then 20.1° to the right and finally 20.1° to the left. The number of saccades greater than 1° (square wave jerks) that intruded upon steady fixation were counted. A frequency higher than 10/min was considered abnormal (Herishanu and Sharpe, 1981).

Smooth pursuit was evoked by a target moving on a cylindrical screen with an array of 512 red gallium-arsenide LEDs of wavelength 635 nm. Each diode subtended a visual angle of 0.14° at a viewing distance of 1 m. Length of the screen was 1.305 m. If viewed from the distance of 1 m, sinusoidal stimuli had an amplitude (measured from the centre of the screen) of 10.5° (peak-to-peak amplitude of 21°). With this procedure, a smooth pursuit eye movement is evoked at 0.3 Hz and 0.5 Hz cycle frequencies in normal subjects (Fig. 1). Analysis was performed on eight cycles. Catch-up saccades were removed from the eye

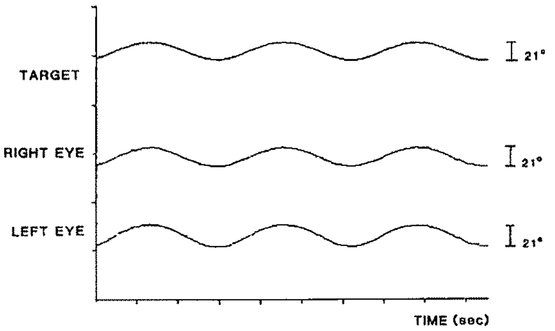

FIG 1. Normal smooth pursuit in a control person

signal as follows. After digitization a velocity signal was derived from the position signal by a two-point central difference algorithm. This was done for target as well as eye signals. As saccades have higher velocites and acceleration than smooth pursuit eye movements, the beginnings and ends of saccades could be identified on the basis of an acceleration threshold. For these periods a new velocity signal was formed by interpolation between the beginning and end points. If the interpolation window is chosen larger than strictly necessary, the threshold level is not critical. The resulting velocity signal represents pursuit without catch-up saccades. When eye velocity is plotted as a function or target velocity over eight sinuses, a linear relationship is found; the slope of this line represents smooth pursuit eye velocity (without catch-up saccades) divided by target velocity and is taken as smooth pursuit gain (Fig. 2). Smooth pursuit gains of patients were compared with values of 10 sex- and age-matched controls.

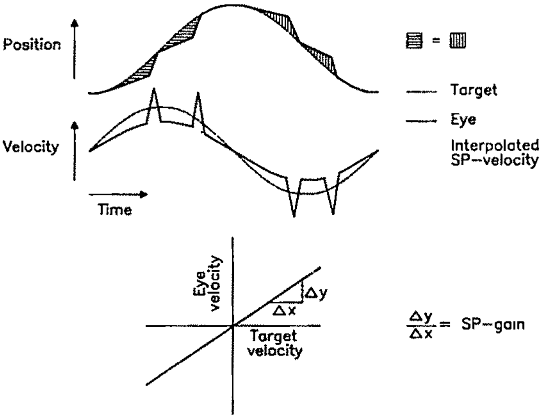

FIG. 2. Illustration of how smooth pursuit gain was calculated A velocity signal was derived from the position signal by differentiation. This was done for target as well as eye signals. As saccades have higher velocities and acceleration than smooth pursuit eye movements, the beginnings and ends of saccades could be identified on the basis of an acceleration threshold. For these periods a new velocity signal was formed by interpolation between the beginning and end points. The resulting velocity signal represents pursuit without catch-up saccades. When eye velocity is plotted as a function of target velocity, a linear relationship is found, the slope of this line represents smooth pursuit eye velocity (without catch-up saccades) divided by target velocity and is taken as smooth pursuit gain.

PATIENTS

Ten patients with MD, six males and four females, were studied. Age ranged from 22 to 71 yrs (mean ± SD, 45.4 ± 16.3). Duration of clinical symptoms varied from 3 to 22 yrs (mean ± SD, 12 ± 7). Diagnosis of MD was based on typical clinical features and a positive family history. Patients were examined neurologically before the study and graded according to the degree of weakness, grip-myotonia, hypersomnia, loss of initiative and ptosis (Table 1). Visual acuity was 0.3 in patient 4 and at least 0.5 in the others. Six patients had been treated surgically for cataract in the past. Patient 3 was taking ephedrine (50 mg daily) to alleviate hypersomnia,the others did not take drugs. Controls were 10 sex-matched healthy individuals, age range 22–71 yrs (mean ± SD, 44.8 ± 15.7). All patients and controls gave informed consent.

RESULTS

Smooth pursuit gain was abnormally low in our patient group as compared with controls for both stimulus frequencies (Table 2). Figure 1 illustrates a normal smooth pursuit eye movement evoked in a control person; Fig. 3 illustrates decreased smooth pursuit gain with catch-up saccades in a patient. Individual smooth pursuit gain values of the patients are also shown in Table 2. There was no relationship between sex, duration of disease, degree of myotonia, degree of weakness on the one hand and smooth pursuit gain on the other (*see* Tables 1, 2).

There were no statistically significant differences in saccadic latency between patients (245 ms ± 28.5 SD) and controls (233 ms ± 19.6 SD).

TABLE 1 DATA FOR 10 PATIENTS WITH MYOTONIC DYSTROPHY

Patient no	*Sex*	*Age (yrs)*	*DD (yrs)*	*Wk*	*My*	*HS*	*LI*	*PT*
1	F	47	5	1	1	±	−	−
2	M	22	19	2	1	++	++	+
3	F	26	3	1	1	+	+	+
4	M	37	9	2	1	++	++	−
5	M	71	15	2	1	++	++	−
6	M	50	4	2	1	±	±	+
7	F	54	22	3	1	++	+	−
8	F	32	19	2	1	−	−	−
9	M	46	9	3	2	++	++	−
10	M	63	11	1	1	±	+	−

DD = duration of disease, Wk = grade of weakness, My = myotonia, HS = hypersomnia, LI = loss of initiative, PT = ptosis Weakness grade 1 = mild, hardly interfering with daily activities, 2 = symptomatic, interfering with some activities, 3 = moderate, interfering with many activities Moyotonia grade: 1 = mild, interfering with some activities, 2 = severe, interfering with many activities. Hyersomnia and loss of initiative grade − = absent; ± = mild, + = moderate; ++ = severe. Ptosis − = absent, + = present.

TABLE 2 SMOOTH PURSUIT GAIN IN 10 PATIENTS WITH MYOTONIC DYSTROPHY

		Gain	
Patient	*Age*	*0.3 Hz*	*0.5 Hz*
1	47	0.65	0.20
2	22	0.89	0.45
3	26	0.76	0.81
4	37	0.45	0.51
5	71	0.80	0.78
6	50	0.81	0.80
7	54	0.89	0.45
8	32	0.82	0.84
9	46	0.68	0.60
10	63	0.92	0.76
Mean	44.8	0.76*	0.60**
SD	15.7	0.14	0.26
Controls			
Mean	45.4	0.93	0.89
SD	16.3	0.05	0.07

*$P < 0.005$ (Wilcoxon test), **$P < 0.01$ (Wilcoxon test)

Fixation was normal in patients and controls, and occurrence of square wave jerks during steady fixation was within normal limits.

Ages of patients and controls did not differ statistically (Table 2).

DISCUSSION

We examined smooth pursuit in 10 MD patients. Smooth pursuit gain was decreased in our patients as compared with controls. In other reports on smooth pursuit in MD

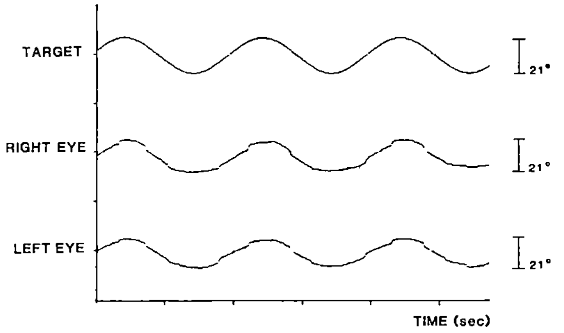

FIG 3 Decreased smooth pursuit gain in patient 7 (frequency = 0.5 Hz) with numerous catch-up saccades

(Oohira *et al.*, 1985; Ter Bruggen *et al.*, 1990), smooth pursuit was found to be interrupted by catch-up saccades. Nevertheless, these authors found normal smooth pursuit gain in their MD patients (Ter Bruggen *et al.*, 1990) even if catch-up saccades were abundant (Oohira *et al.*, 1985). This is surprising, since catch-up saccades are the hallmark of defective smooth pursuit. In these studies, smooth pursuit was evoked by a constant velocity ramp movement of the target, and gain was measured in uninterrupted parts of the smooth pursuit signal between catch-up saccades (Oohira *et al.*, 1985; J.P.Ter Bruggen, personal communication). With our software, gain (eye position/ target position) is calculated by comparing smooth pursuit eye velocity after removal of catch-up saccades with target velocity. This way of analysis takes into account the two characteristics of deficient smooth pursuit: decreased smooth pursuit eye velocity as well as the occurrence of catch-up saccades. It allows comparing eye velocity signal with target velocity signal over longer, standardized periods (eight cycles in our patients) instead of analysing only periods which are selected by visual inspection.

Smooth pursuit gain decrease in our patients was not an aspecific result of lack of attention. Hypersomnia and loss of initiative are common in MD (Harper, 1989) and were reported by eight of our 10 patients. However, fatigue or lack of attention, severe enough to cause smooth pursuit abnormalities would be expected to prolong latencies of saccadic eye movements (Leigh and Zee, 1991). In accordance with Ter Bruggen *et al.* (1990), we found normal saccadic latencies. Alertness during saccadic testing, of course, does not guarantee alertness during smooth pursuit, but since smooth pursuit was investigated before saccades, we assume that fatigue cannot explain the smooth pursuit abnormalities in our patients.

It is not known how different fibre types in extra-ocular muscles contribute to smooth pursuit gain. Extra-ocular muscles contain large diameter twitch fibres, subserving saccadic eye movements, and small diameter twitch fibres, intermediate fibres and tonic fibres probably subserving maintenance of eccentric gaze (Keller and Robinson, 1972; Spencer and Porter, 1988; Haruta *et al.*, 1989; Kaminski *et al.*, 1990). In MD, peak velocity of saccadic eye movements has been found to be decreased (Ter Bruggen *et al.*, 1990), arguing in favour of myopathy of large diameter twitch fibres. However, it is less likely that extra-ocular myopathy explains smooth pursuit gain decrease in our patients. First, no relation was found between degree of grip-myotonia or degree of muscular weakness on the one hand and smooth pursuit gain decrease on the other.

Secondly, patients were able to make catch-up saccades that are of much higher velocity than smooth pursuit. This argues against an eye muscle paresis as the cause of smooth pursuit velocity decrease. Thirdly, fixation of targets in eccentric position of 20° was normal. Although we cannot be sure, this makes myopathy of small diameter twitch fibres, intermediate fibres or tonic fibres a less likely explanation for abnormalities of smooth pursuit eye movements in our MD patients. For these reasons, our study provides evidence for central nervous system abnormalities responsible for defective smooth pursuit in patients with MD. Descending pathways involved in execution of smooth pursuit eye movements pass in periventricular white matter in monkeys and possibly in humans also (Thursten *et al.*, 1988; Tusa and Ungerleider, 1988). Therefore, our results are in accordance with recent reports (Glantz *et al.*, 1988; Huber *et al.*, 1989) of periventricular white matter abnormalities on MRI scan in MD patients.

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STRUCTURE AND FUNCTION OF NEUROMUSCULAR JUNCTIONS IN THE *VASTUS LATERALIS* OF MAN

A MOTOR POINT BIOPSY STUDY OF TWO GROUPS OF PATIENTS

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SUMMARY

The properties of neuromuscular junctions (NMJs) in the *vastus lateralis* of man have been studied in motor point biopsy samples and compared with those reported for lower vertebrates (frogs and mice). The patients studied had no convincing evidence of a primary disturbance of neuromuscular transmission or other neurogenic component. Morphological studies were made using a variety of methods at the light- and electron-microscope levels. The size of the presynaptic nerve terminal and the area of postsynaptic specialization were smaller, relative to the size of the muscle fibres, than in the lower vertebrates. In contrast, the extent of postsynaptic folding was greater. Intracellular recordings from single muscle fibres showed that the duration of synaptic currents was longer than in most other vertebrates so far studied and that the number of transmitter 'quanta' released by a single nerve impulse, about 20, was lower, probably reflecting the small size of the presynaptic terminals. The hypothesis is discussed that in man, a relatively weak effect of transmitter on the muscle fibre surface is amplified by voltage-dependent sodium channels which have been shown in the rat to be concentrated in the depths of the synaptic folds. The implications of this hypothesis for the interpretation of pathological findings in myasthenic syndromes are also discussed.

INTRODUCTION

Numerous diseases of the neuromuscular junction (NMJ) have been defined including myasthenia gravis, the Lambert-Eaton myasthenic syndrome and a variety of other myasthenic syndromes (Engel, 1986; Vincent *et al.*, 1989; Engel *et al.*, 1990). From what is already known, it seems likely that many more such conditions occur and remain to be characterized. In addition, there is evidence that NMJs may be involved in other conditions, such as motor neuron disease, where the NMJ is probably not the primary site of attack by the disease process (Barwick and Fawcett, 1988). The design of rational treatment for the impairment of transmission in these conditions depends on a detailed understanding of the process of neuromuscular transmission at human NMJs and knowledge of the nature of the underlying cellular and molecular abnormalities in individual patients.

Clinical neurophysiological studies, particularly those using single fibre EMG (SFEMG) techniques, are essential tools in recognizing defects of neuromuscular

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transmission. However, it is not possible to define the cellular or molecular sites of the disease process from such studies. For this, detailed studies of isolated biopsy samples using a combination of physiological, anatomical and molecular approaches have been very useful. Previous studies of this kind have generally been made on samples of intercostal muscles (e.g. Elmqvist and Quastel, 1965; Cull-Candy *et al.*, 1979, 1980; Engel *et al.*, 1990). These have the advantage that intact muscle fibres can be obtained and that control material from patients with no evidence of neuromuscular disease can be obtained during chest surgery. As a muscle for routine diagnostic purposes, however, the dangers associated with both preliminary EMG studies and subsequent sampling by biopsy make the intercostals less than ideal. As an alternative, we have been studying NMJs in samples of *vastus lateralis* (*VL*). This has been a preferred muscle for diagnostic biopsy in Newcastle for some time and there is a good background of information about the normal structure and function (e.g. Lexell *et al.*, 1986; Stålberg *et al.*, 1989) and the histopathology of this muscle in many neuromuscular diseases. Removing biopsy samples of *VL* requires cutting the muscle fibres at both ends and the resulting depolarization of the muscle fibres means that action potentials and contractions are reduced or abolished (Barstad, 1962). It is therefore possible to study local synaptic events and the quantal release of transmitter from the motor axon terminals directly. This avoids the use of blocking drugs and of the indirect methods for estimating transmitter release used by others (Elmqvist and Quastel, 1965; Engel *et al.*, 1990).

This paper is the first in a series dealing with the properties of NMJs in human *VL*. In it, we describe NMJs in two groups of patients in whom no evidence of neurogenic abnormality was found by routine EMG or histopathological studies. One group contained patients with muscle pain or myoclonic epilepsy in whom no clear abnormality of muscle or nerve was detected by routine investigations. The other contained patients with various forms of adult onset myopathies. These two groups of 'non-neurogenic' patients form a growing reference panel against which we will compare other groups of patients. In subsequent papers, we will describe NMJs in patients with motor neuron disease and a variety of myasthenic syndromes.

Some of the results described here have been reported previously in abstract form (Fawcett *et al.*, 1987; Slater and Walls, 1987).

MATERIALS AND METHODS

Patient selection

The patients described in this report were part of an ongoing study of the structure and function of the NMJ in neuromuscular diseases which has been approved by the Joint Ethical Committee of the Newcastle Health Authority and the University of Newcastle upon Tyne. All patients included here were undergoing a muscle biopsy for diagnostic purposes. Details of the patients are presented in Table 1. All but one was in the range of 24–44 yrs of age. One patient was only 14 yrs; in none of the measured variables reported here did the values for this patient differ from those of the group as a whole by more than 1.5 times the standard deviation.

Diagnosis. A full history, clinical examination and a functional assessment were performed at the time of biopsy. On the basis of the clinical features, the results of neurophysiological studies (including nerve conduction studies and concentric needle electromyography, CNEMG) and appropriate biochemical investigations (including measurements of serum creatine kinase, CK), a diagnosis was established by the responsible physician, independent of the findings of the present study. In all the patients, nerve conduction was normal.

TABLE 1 PATIENT SUMMARY

Patient no	*Sex*	*Age (yrs)*	*CK (units)*	*Diagnosis/condition*
Group 1				
17	F	38	47	Muscle pain
30	M	29	158	Muscle pain
37	F	30	94	Muscle pain
38	M	41	48	Muscle pain
39	F	14	–	Dementia and myoclonic epilepsy
Average		30.4	87	
SD		10.5	52	
n		5	4	
Group 2				
13	M	33	801	non-Xp21 muscular dystrophy*
15	F	44	40	non-Xp21 muscular dystrophy
16	M	24	3000	Xp21 muscular dystrophy (Becker)
31	M	31	1388	McArdle's disease
Average		33.0	1307	
SD		8.3	1256	
F-test		ns	$P < 0.001$	
t test		ns	ns	
All				
Average		31.6		
SD		9.1		
n		9		

*On basis of immunocytochemistry and immunoblotting

In four patients, who complained of muscle pain of unknown origin, CK levels were normal and CNEMG findings were normal or revealed only mild, non-specific changes. A fifth patient had a dementing illness associated with myoclonic epilepsy and was taking sodium valproate at the time of the biopsy procedure. While none of these patients was suspected of having a primary disturbance of muscle or its innervation, biopsy samples were taken to examine the possibility of a metabolic disorder. For the purposes of our study, these mildly affected patients were placed in Group 1.

Of a further four patients, three had markedly elevated CK levels and CNEMG findings consistent with a necrotizing myopathy. One of these was subsequently proved to have Becker muscular dystrophy and a second, McArdle's disease. These patients were all considered to have a primary myopathic condition uncomplicated by any significant abnormality of innervation and were placed into Group 2. A fourth patient (MPB no. 15) had less severe symptoms but was included in Group 2 because the CNEMG findings suggested a mild myopathic disorder.

The nine patients reported here were all of those in our current series who had no evidence of a primary neurogenic disturbance or of an abnormality of neuromuscular transmission and from whose biopsy samples adequate data were obtained both from *in vitro* studies of neuromuscular transmission and from light and electron microscopy

Electromyography (EMG)

For diagnostic purposes, motor and sensory nerve conduction velocities were measured in all but one patient and a detailed CNEMG examination was performed. These subjects also had a single fibre EMG (SFEMG) study (Stålberg and Trontelj, 1979) performed in the right *VL* in addition to other muscles. Recordings made during voluntary contraction were stored on tape (Neal Ferrograph) for subsequent measurement of jitter, which is expressed as mean consecutive difference (MCD).

Jitter measurements of less than 5 μs were excluded, as such recordings are probably derived from split muscle fibres and therefore do not reflect the state of transmission at NMJs. Recordings showing irregular discharge patterns and interpotential intervals exceeding 4 ms were also excluded because of the likely contribution to jitter by short-term variations in the propagation velocity of the muscle fibre action potential (Stålberg and Trontelj, 1979).

Motor point biopsy procedure

The muscle biopsy was obtained from the left *VL*. The endplate region was located initially by finding the area of the muscle which had the lowest electrical threshold for contraction (typically 3 mA with a pulse duration of 0.05 ms) in response to percutaneous stimulation with a standard EMG bipolar surface electrode. The motor point was usually situated at the junction between the middle and lower thirds of the *VL* and slightly lateral to the midpoint of its fibres.

The biopsy was removed under local anaesthetic (1% lignocaine), taking great care to avoid infiltrating the muscle itself with anaesthetic. After exposing the surface of the muscle, a sterile bipolar stimulating electrode was used to localize the motor point more accurately.

Two bundles of muscle fibres, approximately 6×0.5×0.5 cm centred on the motor point, were removed and immediately placed in a solution with the following composition: NaCl 138.8 mM; KCl 4 mM; KH_2PO_4 1 mM; $NaHCO_3$ 12 mM; $CaCl_2$ 2 mM; $MgCl_2$ 1 mM; glucose 11 mM) gassed with 95% O_2/5% CO_2 (Liley,1956). The wound was then closed. There was no evidence of reduction in the power of the *VL* in any of the patients when examined immediately, and 6 wks, after the procedure.

Of a total of 43 biopsies obtained by this method, from patients with a variety of conditions, we found NMJs in 39 (91%) by either physiological or morphological criteria. We were able to obtain functioning nerve-muscle preparations from 35 of the biopsies (81% of the total).

Structural studies

Histology and histochemistry of muscle fibres

One portion of the muscle was quick frozen in Arcton or isopentane cooled in liquid nitrogen. Transverse frozen sections (10 μm) were stained with haematoxylin and eosin. To determine the major histochemical fibre types, adjacent sections were stained with a histochemical reaction to demonstrate myofibrillar ATPase, after preincubation at pH 9.5, 4.6 and 4.3 (Dubowitz, 1985).

Intramuscular nerves

Both silver impregnation and methylene blue staining were used to study the course and branching pattern of the intramuscular nerves. In successful methylene blue preparations, the preterminal axons could often be traced to the main intramuscular nerve trunks but the details of the axon terminals were not always clear. The silver-cholinesterase preparations were generally more reliable and provided a complementary view; the axons were less consistently stained but the terminals were usually clearer (Fig. 1c,D). Useful information on nerve branching could often be obtained from both types of preparation, and we have pooled the data from the two methods in Table 3.

Methylene blue. A bundle of muscle fibres was immersed in 0.02% methylene blue in gassed Liley's solution at 37°C for 15–30 min. After thorough washing, the preparation was fixed in saturated ammonium molybdate for 1–2 h, washed very thoroughly and then cleared in benzoyl benzoate (Waerhaug and Korneliussen, 1974).

Silver-cholinesterase. A bundle of muscle fibres was fixed in 4% paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.2 at room temperature for about 90 min. It was washed overnight in the same buffer and stained using the method of Karnovsky and Roots (1964), slightly modified as follows, to demonstrate cholinesterase activity.

The washed bundle of muscle fibres was incubated with the substrate (acetylthiocholine iodide) at a concentration of 0.25 mg in 10 ml of medium for 1 h and then at a concentration of 5 mg in 10 ml for a further 15 min. After washing, a portion was teased into single fibres which were mounted in glycerol for morphometric studies of the subneural apparatus. The remainder was further impregnated with silver by the method of Tsuji and Tobin-Gros (1980), slightly modified in that the incubation was carried out at 37°C. Bundles of 10–20 fibres were teased apart, washed and mounted in glycerol.

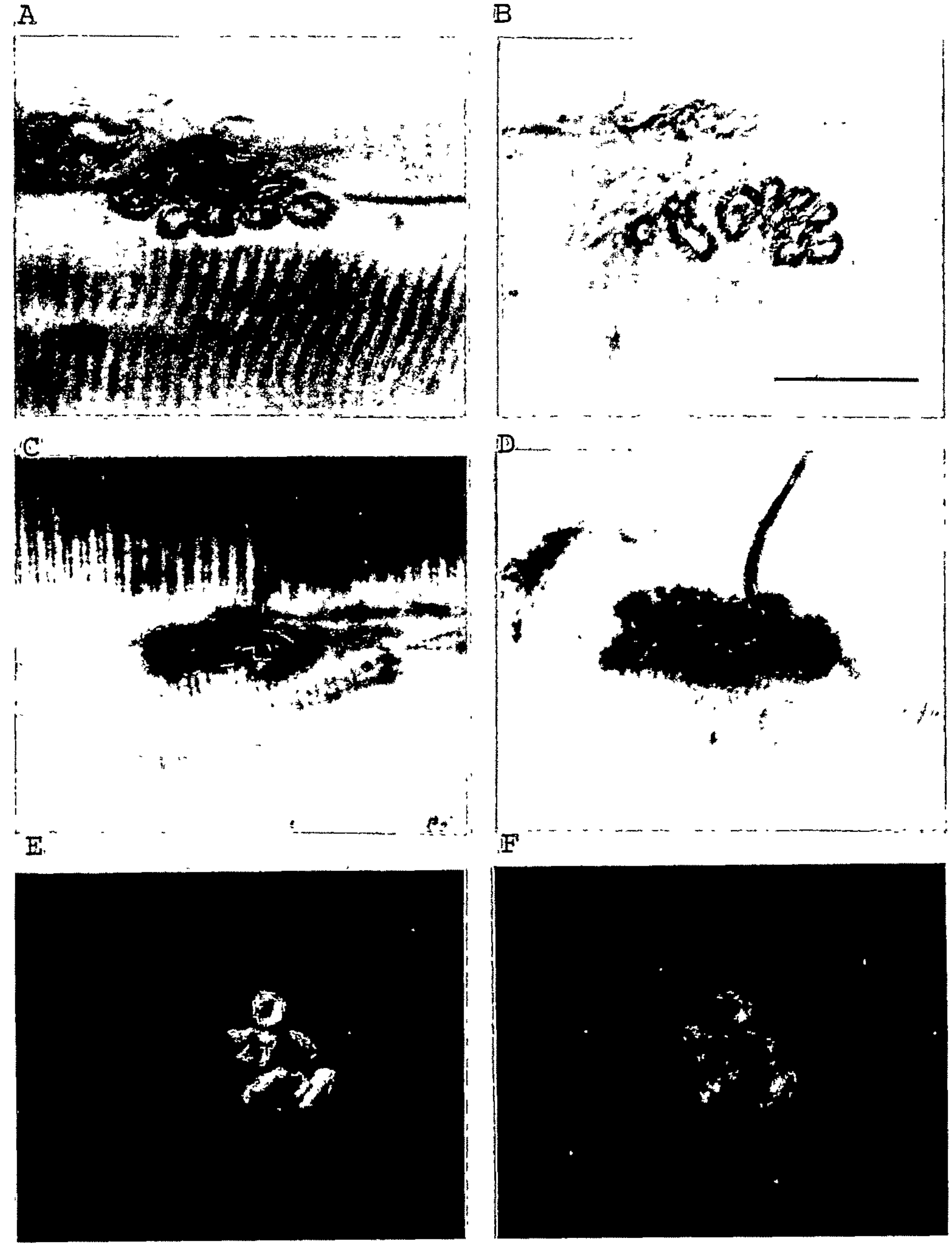

FIG 1 Morphological features of NMJs in human *VL*. A, B illustrate two NMJs in which AChE activity is demonstrated histochemically. The folds of the subneural apparatus can just be seen in these whole mounts of single fibres C, D, presynaptic motor axon terminals in *VL* as seen following silver-cholinesterase staining in teased bundles of muscle fibres. Each terminal branches several times and ends in an expanded bouton E, F show the distribution of AChRs and AChE, labelled with rodamine-α-BgTx and fluorescein-labelled antibody against AChE, respectively. Scale bar = 25 μm

Postsynaptic molecules

Acetylcholine receptors (AChRs). Rhodamine-conjugated α-bungarotoxin (R-α-BgTx) was prepared and used as described by Slater (1982). Bundles of unfixed muscle fibres were labelled for 4 h at room temperature, washed in several changes of Liley's solution for a total of 2 h and then fixed in 1% paraformaldehyde in 100 mM phosphate buffer, pH 7.2 for 30 min. After thorough washing the position of the NMJs was determined under the fluorescence microscope. Small bundles containing NMJs were then teased apart for more detailed observation or labelling for acetylcholinesterase (AChE) (*see below*).

[^{125}I]α-bungarotoxin binding. The number of AChRs per NMJ was estimated in two biopsies (nos 37, 38) from studies of [^{125}I]α-bungarotoxin ([^{125}I]α-BgTx) binding. Small bundles of muscle fibres containing visible intramuscular nerve branches were incubated for 4 h at room temperature in gassed Liley's solution containing 0.5×10^{-8} M [^{125}I]α-BgTx (Amersham, 200–300 Ci/mmol). The samples were then washed for 2 h in repeated changes of Liley's solution, fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 1 h and then washed overnight in Liley's solution at 4°C.

The following day the samples were teased into smaller bundles of 10–20 fibres and the region containing NMJs was identified by the demonstration of AChE activity (Karnovsky and Roots, 1964). From each of 10–20 such bundles, the region containing the NMJs (usually about 0.5 mm long) was cut out together with an adjacent region of the same length. Radioactivity in each piece was determined by gamma counting. Finally, all the pieces were mounted in glycerol and examined in the microscope at a magnification of ×400 to count the number of NMJs.

From these data, the number of [^{125}I]α-BgTx binding sites/NMJ was calculated.

Acetylcholinesterase. Small muscle fibre bundles were incubated overnight at 4°C in a rabbit serum raised against rat brain AChE (Marsh *et al.*, 1984; a kind gift of Professor J. Massoulié, Paris), at a dilution of 1:1000 in phosphate buffered saline (PBS) containing 0.1 M lysine and 3% bovine serum albumin. After washing at room temperature, the bundles were incubated for 4–6 h at room temperature in 1:100 fluorescein-conjugated swine anti-rabit serum (Dako). After washing overnight at 4°C, the bundles were mounted in glycerol.

Immuno-labelling of AChE was usually combined with R-α-BgTx labelling of AChRs in the same preparations so that the relative distributions of the two proteins at individual NMJs could be studied.

Ultrastructure

A portion of the biopsy sample in which fine intramuscular nerve branches could be seen clearly was fixed in 5% glutaraldehyde in phosphate buffer (100 mM, pH 7.2) containing 70 mM sucrose for 90 min at room temperature and then washed. The muscle was then teased into bundles of 10–20 fibres. To localize NMJs, cholinesterase activity was demonstrated histochemically using the method of Strum and Hall-Craggs (1982). After washing overnight at 4°C, the bundles were osmicated, dehydrated and embedded in Araldite using standard procedures.

Regions containing NMJs were identified by the appearance of AChE staining in semithin (0.5 μm) sections. Ultrathin sections were then cut and stained with uranyl acetate and lead. Sections were examined in a JEOL 1200 microscope. All NMJs found were photographed at ×10 000 and subsequently analysed. Photomontages of each NMJ, at a final magnification of ×40 000, were made for morphometric analysis.

Morphometric analysis

Measurements of features of interest, at both the light and electron microscope levels, were made using a digitizing tablet and associated software (Kontron VIDEOPLAN, Kompira IMAGAN2). Light microscope preparations were measured directly, using a drawing tube and illuminated cursor, at a microscope magnification of ×1000. Contours of interest in electron micrographs were traced onto acetate sheets for subsequent measurement.

Muscle fibre diameters. From photographs of serial sections, reacted to demonstrate myofibrillar ATPase, a population of 200 muscle fibres was defined and each fibre classified according to its metabolic fibre type. The fibre diameter was taken to be the lesser diameter of an ellipse with the same moment of inertia as the cross-section of the fibre (Song *et al.*, 1963).

Innervation pattern. The distribution of intramuscular nerves was examined in methylene blue and silver-cholinesterase preparations. Motor axons were followed as far back as possible, usually 100–200 μm, from the NMJs toward the intramuscular nerves. The innervation of particular muscle fibres was said to be 'collateral' when it could be seen to arise from an axon that branched distal to its exit from an intramuscular nerve bundle. The functional terminal innervation ratio, the ratio of the number of axons which have left the intramuscular nerve bundle to the number of muscle fibres innervated by those axons (FTIR; Coërs and Woolf, 1959), was calculated from such observations.

Each NMJ was examined for the presence of sprouts, defined as unmyelinated outgrowths not leading to a well-defined presynaptic terminal but often ending in a swelling resembling a growth cone (Barker and Ip, 1966). If present, sprouts were classified as nodal, preterminal or ultraterminal, depending on their site of origin from the axon. Generally 50–100 NMJs stained by each method were examined and classified. The results from the two methods of staining were pooled for analysis.

Subneural apparatus. The total area of the muscle fibre surface occupied by the subneural apparatus was determined from teased single fibres in which AChE activity has been demonstrated. The area of intense staining was carefully outlined at a final microscope magnification of ×1000. The diameter of the muscle fibres, defined as their maximum width at the level of the NMJs, and the length of the area of AChE activity, measured along the length of the muscle fibre, was also recorded. In most samples, 20–25 (mean 23) NMJs were analysed.

Ultrastructure. Each section through a NMJ normally contained one or more axonal profiles and one or more distinct regions of postsynaptic folding. For each NMJ observed, the following features were measured and their values are listed in Table 5: (i) the number of separate axon terminal profiles (#NT); (ii) the total area of the axon terminals (NTA); (iii) the total length of the axon terminal perimeter in direct contact with the muscle fibre (PreL); (iv) the number of distinct regions of postsynaptic folding (#FR); (v) the total surface length of the subneural apparatus measured along the tops of the folds ('surface length', SurfL); (vi) the total length of postsynaptic membrane including the folds ('fold length', FoldL); (vii) the total area containing the postsynaptic folds as defined by Engel and Santa (1971) (PostA).

From these measurements, several derived values were calculated for each NMJ. The means of these values for each patient group and for the pooled data are listed in the upper part of Table 6. In the lower part of the table, comparable values from several other studies of human NMJs are listed: (i) the mean area of the axon terminal profiles (mNTA = NTA/#NT); (ii) the mean presynaptic length per nerve terminal profile (mPreL = PreL/#NT); (iii) the mean number of axon profiles per folding region (#NT/#FR); (iv) the fraction of the surface length in contact with axon terminals ('occupancy' = SurfL/PreL); (v) the ratio of the fold length to the surface length ('folding index' = FoldL/SurfL), a measure of the local intensity of folding; (vi) the density of folded membrane within the postsynaptic area ('Fold density' = FoldL/PostA); (vii) the mean fold length per axon terminal profile (mFoldL = FoldL/#NT); (viii) the mean postsynaptic area per axon terminal profile (mPostA = PostA/#NT); (ix) the ratio of the fold length to the presynaptic length (FoldL/PreL). An average of 10 NMJs were analysed from each biopsy sample

Functional studies

Nerve-muscle preparations

Under a dissecting microscope, one or two small muscle bundles, each innervated by an intact branch of the intramuscular nerve, were isolated for intracellular recording. The nerve-muscle preparations were pinned out in a Perspex bath (volume 7.5 ml) and continuously perfused with fresh Liley's fluid at a rate of 3 ml/min at room temperature (21–23°C). For stimulation, the nerve was drawn up into a suction electrode of suitable size and stimulated with pulses of 0.1 ms duration

To block muscle fibre action potentials and contraction, the muscle bundles were cut further to a final length of 2 cm (Barstad, 1962; Glavinović, 1979*a*). Such preparations remained stable and good recordings of synaptic events could usually be obtained for up to 24 h.

Intracellular recording

Voltage and current transients were recorded using an Axoclamp-2 (Axon Instruments), in either 'bridge' or '2-electrode voltage clamp' mode. Conventional glass microelectrodes with resistances of about 5 MΩ were used; voltage electrodes were filled with 3 M KCl and current passing electrodes with acidified 2 M sodium acetate.

After amplification, all signals were recorded on magnetic tape (RACAL Store 4, 7½ inches/s, bandwidth 0–5 kHz) for subsequent analysis.

Recordings of spontaneous and evoked synaptic events were made from an average of 15 NMJs in each biopsy sample. Synaptic potentials were recorded at all NMJs studied, but currents were only recorded at some.

Cable properties

To measure the input resistance of the muscle fibres, two electrodes were inserted less than 100 μm apart. Input resistance was calculated as the ratio of the final amplitude of the voltage transient resulting from the injection of a 20 ms long hyperpolarizing current pulse to the amplitude of that pulse (usually 30 nA). An estimate of the time constant was obtained from the time taken for the voltage transient to reach 84% of its final value (Hodgkin and Rushton, 1946).

Synaptic potentials

Endplate potentials (EPPs) were recorded at a stimulus frequency of 1 Hz. In spite of detailed searching, few EPPs were observed with a rise time (time to go from 10% to 90% of peak amplitude) of less than 0.75 ms. Recordings were considered 'focal', and hence acceptable, if the rise time was not more than twice this value. Because the resting potential of the muscle fibres was usually less negative than −50 mV, the synaptic potentials were often small. To increase their size, two electrodes were often inserted into each fibre, one being used to pass current to increase the local membrane potential to a value as close to −80 mV as possible without eliciting a muscle fibre action potential.

The frequency of spontaneous miniature EPPs (mEPPs) was rarely more than a few per minute. To obtain an adequate number for analysis, a short train of stimuli (20−100 Hz for 10 s) was given (Cull-Candy *et al.*, 1980). This resulted in a marked rise in mEPP frequency, often initially to as high as 30 s, falling back to resting levels over the next minute or so.

Synaptic currents

Endplate currents (EPCs) were recorded from the same sites as EPPs. Voltage clamping was regarded as satisfactory if voltage changes were reduced to <3% of the unclamped value and if the voltage change in response to a d.c. command was within 1% of the command value.

Endplate currents were usually recorded at a membrane potential of −60 to −80 mV. In many fibres, EPCs were also recorded at several other values of membrane potential to provide information about the reversal potential and the influence of membrane potential on the rate of decay of the EPC (*see below*). Miniature EPCs were recorded at a membrane potential of −80 mV, usually after brief high frequency stimulation to increase their frequency (*see above*).

Analysis of signals

Recorded signals were replayed through a variable active filter (Kemo, type VBF/4) with a frequency cut-off of 5 kHz for voltage signals and 1.5 kHz for current signals. The signals were digitized at a frequency of 12.8 kHz by a CED 1401 (Cambridge Electronic Designs) interface controlled by a BBC microcomputer, programmed to allow manual rejection of artefactual records and to calculate a number of features of the events (Fawcett *et al.*, 1987).

Evoked events. For each EPP and EPC the following features were determined: (i) rise time, defined as the time from 10% to 90% of the peak value; (ii) peak amplitude; (iii) time to decay to half-maximum amplitude; (iv) membrane potential just after the event.

Spontaneous events. The recorded signal was split and one version filtered at 200 Hz and passed through a pulse height discriminator set to trigger the computer. The second version was delayed 2−4 ms before being presented to the computer for analysis. The membrane potential was determined at the beginning and the end of each series of spontaneous events and the mean of these values used in subsequent calculations (*see below*).

Correction of signals. For both spontaneous and evoked events, background noise in the recording system was sampled and half the peak to peak value subtracted from the peak amplitude of each recorded event. All values of peak amplitude were then normalized to a standard membrane potential of −80 mV according to the formula:

$$A_{-80} = A \times (-80/V_{\mathrm{m}}),$$

where A_{-80} = normalized amplitude, A = recorded amplitude, V_{m} = measured membrane potential. The reversal potential was assumed to be 0 mV. No correction was made for the reduction in input resistance resulting from cutting the muscle fibres.

Reversal potential. The reversal potential of the EPCs was either determined directly or estimated by extrapolating the least squares regression line relating EPC amplitude to membrane potential. In the latter case, values were only used if the correlation coefficient was >0.95.

Quantal content. A 'direct' estimate of the mean quantal content of the EPPs and EPCs at each NMJ studied was calculated by dividing the mean amplitude of the evoked response [after correction for 'non-linear summation' in the case of EPPs (Martin, 1955)] by the mean amplitude of the spontaneous events. For comparison, quantal content was also estimated by the 'variance' method according to the following formula:

$$m_{\mathrm{var}} = A^2/\mathrm{Var},$$

where m_{var} = the estimated quantal content, A = mean evoked response amplitude and Var = variance of evoked response amplitude (Martin, 1966). In most fibres, 50–100 events were used for this calculation.

Statistics of transmitter release. The release of transmitter quanta from chemical synapses has often been described in terms of a binomial model in which the mean number of quanta released by a presynaptic action potential is given by the product of n, often interpreted as the number of quanta available for release and p, the probability that any one of those quanta will in fact be released.

Two methods have been widely used to determine these binomial variables; one depends on a statistical analysis of the variance of evoked and spontaneous events in a train, usually at low frequency (e.g. 1 Hz) (*cf* McLachlan, 1978, equation 14), to estimate the probability of release. The other involves extrapolation of the 'run down' of evoked responses during high frequency repetitive stimulation to estimate the total number of quanta in an 'immediately releasable' pool (e.g. Elmqvist and Quastel, 1965; Engel *et al.*, 1990). We have compared the use of both methods.

(i) The value of p was calculated from the values of the amplitudes of 50 or more evoked responses (1 Hz) and spontaneous quantal events according to the formula (McLachlan, 1978):

$$p = 1 - S^2/mx^2 + \sigma^2/x^2$$

where S is the standard deviation of the evoked responses, m is the mean quantal content, x is the mean amplitude of the spontaneous quantal events and σ is the standard deviation of those events. n was then calculated from the relation $m = n \times p$.

(ii) The value of n was estimated from the 'run down' of quantal content in the trains of responses at 20–50 Hz used to increase the frequency of spontaneous transmitter release (Elmqvist and Quastel, 1965; Kamenskaya *et al.*, 1975; Glavinović, 1979*b*; Engel *et al.*, 1990). For this analysis, the first 10 responses were used. In order to avoid undue fatigue of the preparation, only a single train was used for each NMJ studied. Because of the large statistical variation in quantal content from one response to the next in individual trains, we pooled the results for 14 NMJs in three muscles.

For each NMJ, the amplitude of each of the 10 responses was expressed as a fraction of the first. The average value of each response for the 14 NMJs was then calculated and finally multiplied by the mean quantal content, determined at 1 Hz, for all 14 NMJs. The value of n was then estimated as described by Elmqvist and Quastel (1965)

Endplate current kinetics. To analyse the rate of decay of synaptic currents, the digitized events at each NMJ were used to obtain an averaged trace. A single exponential was fitted to the portion of the falling phase from 80% to 20% of the peak value and the decay time constant, τ_{EPC}, was calculated. The correlation coefficient for this fit was almost always >0.99. A similar procedure was used to estimate the decay time constant of the mEPCs.

The value of τ_{EPC} depends on membrane potential (V_{m}) according to the relation:

$$\tau_{\mathrm{EPC}}(V_{\mathrm{m}}) = \tau_{\mathrm{EPC}}(0)e^{-V\mathrm{m}/\mathrm{H}}$$

(Magleby and Stevens, 1972; Cull-Candy *et al.*, 1979). The constant H, which defines the voltage dependence of acetylcholine induced channel closure was estimated from the slope of the least squares regresson line relating $\log\tau_{\mathrm{EPC}}$ to V_{m}. Values were only accepted if the correlation coefficient of this line was >0.95.

Statistics

Numerical data are presented in the text as mean ± standard deviation. Comparisons of variance were made with the F-ratio test and of means with Student's *t* test, using the appropriate form when the variances were significantly different.

RESULTS

Structural studies

Muscle fibres

Fibre types. The proportions of type 1, 2A, 2B and 2C fibres, determined on the basis of histochemically demonstrated myofibrillar ATPase activity, are given in Table 2. There were slightly fewer type 2A and appreciably more type 2C fibres in the patients in Group 2 than in those in Group 1.

Diameter distribution. The mean diameters of muscle fibres in the two patient groups were not significantly different, nor were the diameters of the fibres in the three main histochemically defined groups (Table 2); the mean value for all patients and fibre types was 54.9 μm. In one patient (MPB no. 13) the fibre diameter was substantially larger than in the others (82.1 μm compared with a mean of 53.8 ± 14.1 μm). It will be seen below that several features of the NMJ which are normally related to muscle fibre diameter reflect this fact.

TABLE 2 MUSCLE FIBRE PROPERTIES

Patient no	*Diameter (μm)*					*Proportion (%)*			
	All	*1*	*2A*	*2B*	*2C*	*1*	*2A*	*2B*	*2C*
Group 1									
17	54.0	61.6	56.2	39.0	–	44.0	29.0	27.0	0.0
30	55.7	48.5	58.5	56.1	51.3	33.0	45.0	21.5	0.5
37	62.0	66.8	58.9	55.8	–	47.5	30.0	22.5	0.0
38	51.6	50.2	56.6	49.9	39.3	28.0	30.5	40.5	1.0
39	39.5	42.2	40.0	36.2	39.7	22.5	45.0	25.5	7.0
Average	52.6	53.9	54.0	47.4	43.4	35.0	35.9	27.4	1.7
SD	8.3	10.1	7.9	9.3	6.6	10.6	8.3	7.7	3.0
n	5	5	5	5	3	5	5	5	5
Group 2									
13	82.1	82.3	85.2	55.5	67.4	85.5	11.0	0.5	3.0
15	47.4	58.9	46.4	31.6	60.0	36.5	23.0	32.0	8.5
16	51.3	65.2	77.1	33.3	54.9	16.0	16.0	40.5	27.5
31	50.4	42.0	56.1	51.0	49.4	24.0	34.5	32.0	9.5
Average	57.8	62.1	66.2	42.9	57.9	40.5	21.1	26.3	12.1
SD	16.3	16.7	18.0	12.2	7.7	31.2	10.2	17.6	10.6
n	4	4	4	4	4	4	4	4	4
F-test	ns	ns	ns	ns	ns	ns	ns	ns	$P < 0.05$
t test	ns	ns	ns	ns	$P < 0.05$	ns	$P < 0.05$	ns	ns
All									
Average	54.9	57.5	59.4	45.4	51.7	37.5	29.3	26.9	6.3
SD	11.9	13.2	13.9	10.2	10.2	20.7	11.6	12.1	8.8
n	9	9	9	9	7	9	9	9	9

Intramuscular nerves

The results of these studies are presented in Table 3. The main features of innervation did not differ from those described for other human muscles (e.g. Coërs and Woolf, 1959; Engel, 1986).

TABLE 3 FEATURES OF TERMINAL INNERVATION IN *VL*

Patient no	*Sprouts (%)*	*Collaterals (%)*	*FTIR*
Group 1			
17	0.0	0.0	1.00
30	1.3	13.0	1.14
37	6.0	15.0	1.08
38	6.5	22.0	1.13
39	6.0	21.0	1.13
Average	4.0	14.2	1.10
SD	3.1	8.8	0.06
n	5	5	5
Group 2			
13	11.0	17.0	1.12
15	3.5	10.5	1.11
16	9.0	10.3	1.07
31	15.0	18.0	1.11
Average	9.6	14.0	1.10
SD	4.8	4.1	0.02
n	4	4	4
F-test	ns	ns	ns
t test	ns	ns	ns
All			
Average	6.5	14.1	1.10
SD	4.7	6.7	0.04
n	9	9	9

Collateral innervation. Most muscle fibres were innervated by a single axon which remained unbranched during its course from the intramuscular nerve trunk of origin to the muscle fibre (Fig. 1C,D). In all of the biopsy samples from the two groups of patients, fewer than 25% of the NMJs were innervated by collateral branches (mean 14%). There was no significant difference between the extent of collateral innervation in the two groups of patients.

Functional terminal innervation ratio (FTIR). The FTIR did not differ significantly between the two patient groups (Table 3).

Sprouting. Sprouts were present on not more than 15% of terminal axons in any of the biopsy samples studied.

Terminal innervation

The NMJs in *VL* generally occur on a raised dome of cytoplasm, generally 30–40 μm in diameter and 5–10 μm high (Fig. 1A). Within the NMJ, the motor axon terminal usually branches several times. At most NMJs in samples from both groups of patients, the terminal branching pattern was of the T1 category described by Tuffery (1971); i.e. a single myelinated axon with a number of unmyelinated branches. In silver stained material, these terminal branches often end in swollen boutons 1–2 μm in diameter (Fig. 1C,D).

Postsynaptic molecules

Most of the synaptic dome is occupied by the subneural apparatus, initially defined by Couteaux (1960). This includes deep infoldings of the muscle fibre surface (*see below*) and high concentrations of a number of important synaptic molecules, more or less closely associated with the terminal boutons of the motor axon.

Acetylcholine receptors. Acetylcholine receptors are present in high concentration in the immediate subsynaptic membrane. With the light microscope, using R-α-BgTx, these concentrations appear as one more irregularly shaped, spot-like regions of labelling, each with a smooth, intense outline (Fig. 1E). These often appeared to occupy a much smaller fraction of the muscle fibre surface than in most other mammalian muscles that have been studied so far (e.g. Slater, 1982; Lyons and Slater, 1991). The mean number of $[^{125}I]\alpha$-BgTx binding sites was measured in samples from two patients in Group 1. In each case the number of $[^{125}I]\alpha$-BgTx binding sites was 2.6×10^7/NMJ, similar to that reported by others for human quadriceps (3.4×10^7; Pestronk *et al.*, 1985).

Acetylcholinesterase. The distribution of AChE molecules was compared with that of AChRs at the same NMJs using a double label fluorescence technique (*see* Methods). At all NMJs examined in this way, the two synaptic proteins had very similar distributions (Fig. 1E,F).

Acetylcholinesterase activity was also localized using a histochemical method. The distribution of AChE studied in this way was qualitatively similar to that seen with the immunocytochemical method (Figs 1, 2).

Synaptic area. The extent of the synaptic membrane was estimated using teased single muscle fibres on which AChE activity had been demonstrated histochemically. As previously reported in other species (e.g. Kuno *et al.*, 1971; Harris and Ribchester, 1979), the area of high AChE activity was significantly correlated with the muscle fibre diameter (Fig. 2). There was no significant difference between NMJs in the two patient groups in either the mean area or the mean ratio of NMJ area to the diameter of the teased fibres, which was similar to that measured from frozen sections (Table 4). It can be seen from this table that the sample with the largest synaptic area, patient no. 13, is that one in which the fibre diameter was also greatest (*see above*).

Ultrastructure

General appearance

The ultrastructure of NMJs in *VL* is broadly similar to that in other human muscles (Engel, 1986). Most sections from NMJs in *VL* include one or more regions of highly folded postsynaptic membrane, each associated with a motor axon terminal profile (Fig. 3). In Table 5 we present morphometric data from 90 NMJs from the nine patients. In this table, the entries are the full extent of the values measured in each sectioned NMJ. In Table 6, we present a number of values derived from those measurements,

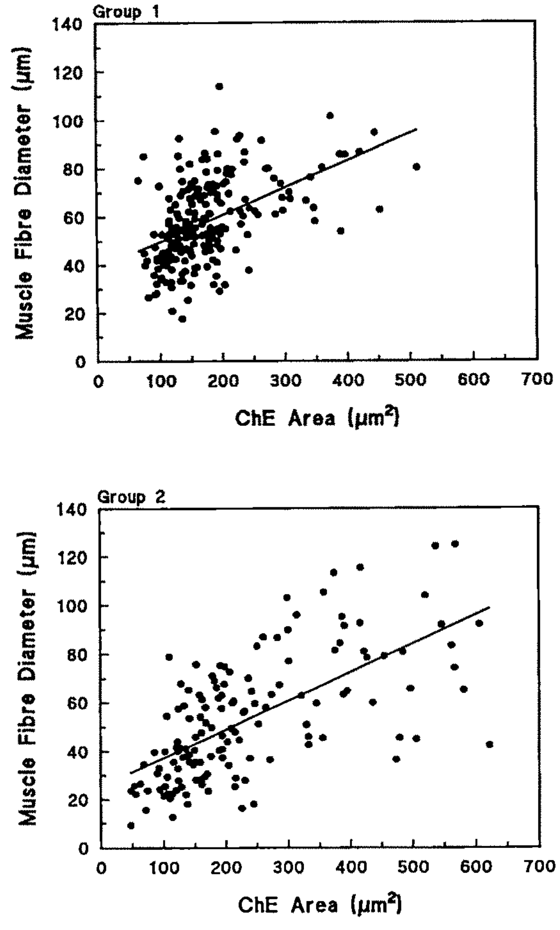

FIG 2. The relationship between NMJ area, measured from the area of high AChE activity in single fibres, and muscle fibre diameter Data are shown separately for NMJs in the two groups of patients. The lines are the least squares regression lines.

such as the extent of various of the features per axon terminal profile. In the bottom part of this table, we compare our values with values reported by others for human NMJs in biceps brachii (BB) and intercostals (IC).

Nerve terminals

In patients of both groups, more than 95% of the 128 regions of postsynaptic folding we examined were in contact with at least one motor axon terminal bouton. Both the mean cross-sectional area of the terminal boutons and the length of their perimeter in immediate contact with the postsynaptic surface of the muscle ('presynaptic length') were considerably less than in IC and slightly less than in BB.

At almost every NMJ, the nerve made contact with only part of the postsynaptic surface; the mean 'occupancy' was about 40% in each patient group. The nerve terminals were covered by extension of the Schwann cell which were surrounded by extracellular matrix. At most NMJs, multiple layers of basal lamina were present in the perisynaptic area rather than a single layer as seen surrounding the muscle fibre and in the junctional cleft (Fig. 3).

TABLE 4 EXTENT OF SYNAPTIC SPECIALIZATIONS OF NMJs IN *VL*

Patient no	*ChE-A* (*μm*²)	*ChE-L* (*μm*)	*Fibre-D* (*μm*)	*ChE-A/Fibre D* (*μm*²/*μm*)	*Occ* (%)	*Total area* (*μm*²)
Group 1						
17	150.4	32.3	54.2	2.77	47.1	70.8
30	290.0	38.8	66.8	4.34	48.3	140.1
37	161.7	31.8	72.8	2.22	34.9	56.4
38	180.5	34.0	64.4	2.80	27.2	49.1
39	137.4	27.6	44.2	3.11	43.4	59.6
Average	184.3	32.9	60.5	3.00	40.2	75.2
SD	61.3	4.1	11.3	0.80	9.0	37.1
n	5	5	5	5	5	5
Group 2						
13	380.4	58.3	76.9	4.95	36.1	137.3
15	153.3	38.0	44.1	3.48	40.3	61.8
16	190.9	47.6	34.7	5.50	47.1	89.9
31	173.7	39.2	58.5	2.97	36.4	63.2
Average	224.6	45.8	53.6	4.2	40.0	88.1
SD	105.0	9.4	18.4	1.2	5.1	35.3
n	4	4	4	4	4	4
F-test	ns	ns	ns	ns	ns	ns
t test	ns	$P < 0.001$	ns	$P < 0.001$	ns	ns
All						
Average	202.0	–	57.4	–	40.1	80.9
SD	80.5	–	14.3	–	7.1	34.7
n	9	–	9	–	9	9

Subneural apparatus

Folding. As a result of folding, the amount of surface membrane associated with the subneural apparatus is increased approximately eightfold (Table 6). The folding index was similar at NMJs from the two patient groups.

Total presynaptic area. The amount of transmitter released from a motor axon terminal is likely to be related to the total area of synaptic contact between nerve and muscle. An estimate of that area can be made by multiplying the synaptic area, determined in the light microscope from measurements of the area of high AChE activity, by the occupancy of that synaptic area, determined in the EM.

Estimates for total presynaptic area are presented in Table 4. The mean value for all the material studied was 81 μm^2. The value for the patients in Group 2 was not significantly different from that for patients in Group 1.

Total area of folded membrane. An estimate of the total area of folded postsynaptic membrane can be made in a similar way to that used to estimate the total area of presynaptic contact. Multiplying the area of the AChE activity (overall mean 202 μm^2) by the folding index (overall mean 8.0) gives a mean value of 1616 μm^2 for the total area of folded postsynaptic membrane per NMJ.

Acetylcholine receptor density. The region of high AChR density is well known to include the tops of the folds and to extend about one-third of the way down the folds. While we have not measured it in detail, it is clear from our images and from the studies

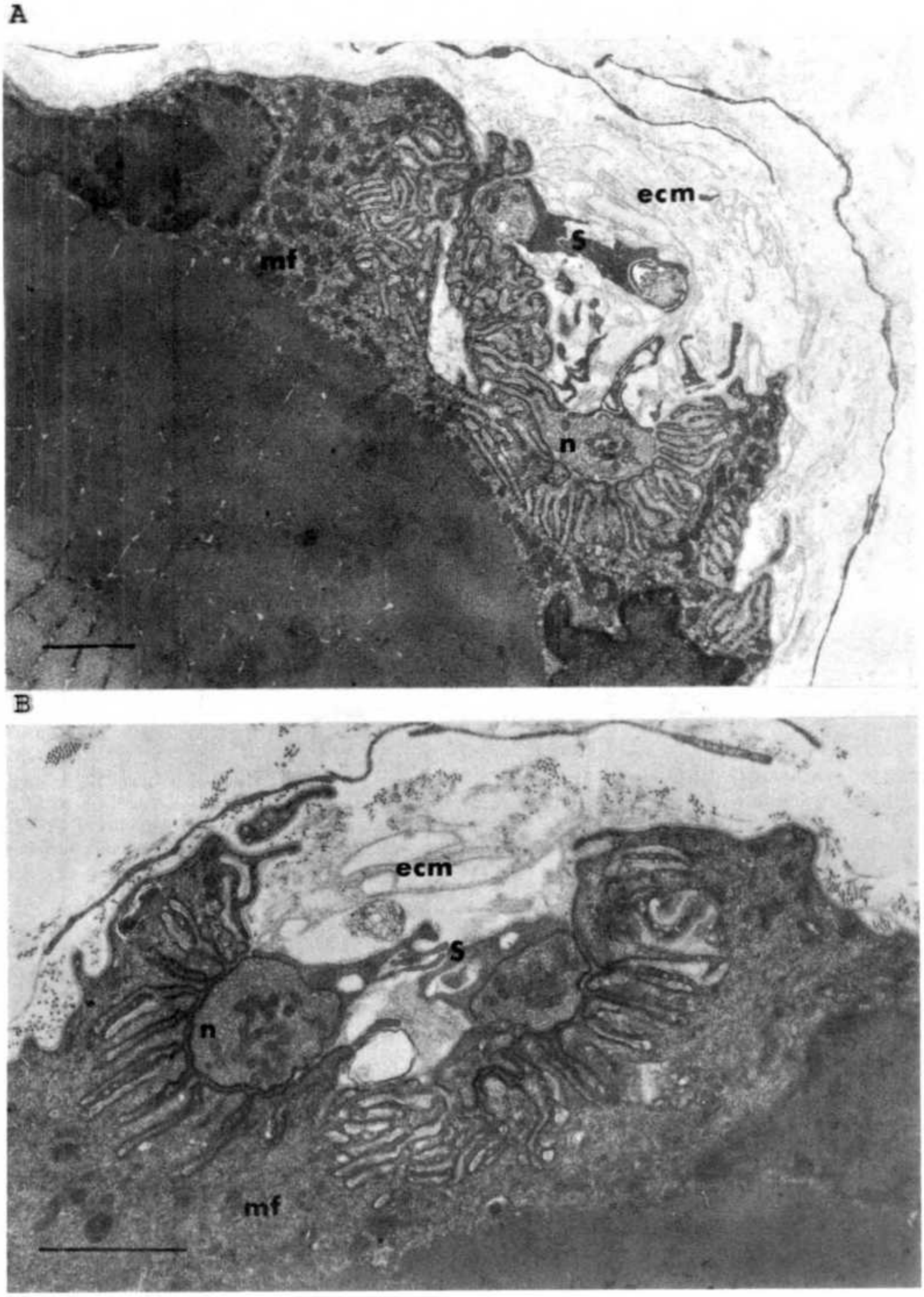

FIG. 3. Ultrastructure of the NMJ in *VL*. Typical examples of the junctional region showing: nerve terminal boutons (n); Schwann cell (S); the highly folded surface of the muscle fibre (mf); multiple layers of extracellular matrix (ecm). Scale bar = 2 μm.

TABLE 5. ULTRASTRUCTURAL FEATURES OF NMJs IN *VL*-MEASURED VALUES

Patient no.	*#NT*	*NTA* (μm^2)	*PreL* (μm)	*#FR*	*SurfL* (μm)	*FoldL* (μm)	*PostA* (μ^2)
Group 1							
17	1.50	2.29	5.39	1.33	13.9	115.0	15.9
30	2.00	3.00	6.65	1.92	15.1	159.1	22.4
37	1.50	1.22	4.16	1.00	11.8	99.6	19.3
38	1.54	2.14	3.55	1.15	14.1	94.2	14.6
39	1.76	3.02	6.58	1.36	16.9	115.0	19.3
Average	1.66	2.33	5.27	1.35	14.4	116.6	18.3
SD	0.22	0.74	1.40	0.35	1.8	25.5	3.1
n	5	5	5	5	5	5	5
Group 2							
13	1.63	2.40	5.01	1.38	13.7	94.4	16.2
15	1.33	1.66	3.17	1.33	10.4	71.8	13.3
16	2.20	2.63	6.57	2.20	14.1	73.9	12.4
31	1.45	3.55	4.27	1.27	12.8	99.8	13.9
Average	1.65	2.56	4.75	1.55	12.8	85.0	13.9
SD	0.38	0.78	1.43	0.44	1.7	14.2	1.6
n	4	4	4	4	4	4	4
F-test	ns	ns	ns	ns	ns	ns	ns
t test	ns	ns	ns	ns	ns	ns	$P = 0.04$
All							
Average	1.66	2.43	5.04	1.44	13.7	102.5	–
SD	0.28	0.72	1.35	0.38	1.9	26.1	–
n	9	9	9	9	9	9	–

TABLE 6. ULTRASTRUCTURAL FEATURES OF NMJs IN *VL*-DERIVED VALUES

Class	*mNTA*	*mPreL*	*#NT/#FR*	*Occ.*	*FI*	*Fold dens.* ($\mu m^2/\mu m$)	*mFoldL* (μm)	*mPostA* (μm^2)	*FoldL/PreL*
VL									
Group 1	1.38	3.15	1.26	0.39	8.1	6.4	69.8	11.0	22.6
Group 2	1.59	2.85	1.03	0.37	6.7	6.1	53.5	8.8	19.0
All *VL*	1.48	3.01	1.18	0.38	7.5	6.2	62.6	10.0	21.0
SD	0.44	0.49	0.17	0.07	1.5	0.8	13.6	1.9	4.6
Intercostals (IC)									
(1)	3.90	–	–	–	–	5.8	61.7	10.6	10.1
(2)	5.00	4.10	–	–	–	6.3	54.7	9.1	13.3
(3)	3.60	5.10	–	–	–	5.7	61.7	11.4	12.1
All IC	4.17	4.60	–	–	–	5.9	59.4	10.4	11.8
SD	0.74	0.71	–	–	–	0.3	4.0	1.2	1.6
Biceps brachii									
(4)	2.80	3.60	–	–	–	6.1	49.6	8.3	13.8

(1) Engel and Santa (1971); (2) Arizono (1984); (3) Wokke *et al.* (1990); (4) Tsujihata *et al.* (1984).

of others that the openings of the folds occur at intervals of about 1 μm. On this basis, the average area occupied by AChRs may be calculated to be approximately 650 μm^2/NMJ. From this, and the estimated number of α-BgTx binding sites per

NMJ (2.6×10^{-7}, *see above*), we calculate that the local site density is approximately $3\times10^{7}/\mu m^{2}$, a value similar to that found by others (e.g. Salpeter, 1987).

The remainder of the folded postsynaptic surface, some 959 μm^{2}/NMJ, is likely to contain a high concentration of voltage-dependent Na^{+} channels (Flucher and Daniels, 1989).

Functional studies

Clinical neurophysiology

Single fibre EMG. Detailed SFEMG studies were made in all patients from the left *VL*.

The fibre density (FD) for individual patients in Group 1 was 1.39 ± 0.31 ($n = 4$) and in Group 2 was 1.60 ± 0.27 ($n = 4$). These values were not significantly different from each other nor did they differ from the mean value of 1.32 ± 0.11 found in eight normal subjects (three females, five males, aged 23–58 yrs, mean 33.6 ± 11.4 yrs).

The mean jitter (MCD) in patients in Group 1 was 33.1 ± 8.1 μs ($n = 4$) which was not significantly different from that in 11 normal subjects (three females, eight males, mean age 36.8 ± 11.6 yrs; mean jitter 26.4 ± 0.19 μs). Jitter in Group 2 was 41.6 ± 10.7 μs ($n = 4$), not significantly different from that in Group 1 but significantly higher than in the normal subjects.

Electrophysiology in vitro

Passive properties. Because all the muscle fibres were cut, accurate values of passive membrane properties of the intact fibres could not be obtained. Initial resting potentials were usually −40 to −60 mV and decreased to −20 to −40 mV after cutting the muscle fibres further to a final length of 2 cm. After such cutting, 85% of the fibres studied had input resistances in the range 100–400 kΩ (mean 255 ± 110 kΩ) and a mean charging time constant of 6.5 ± 2.2 ms. There was a significant correlation between input resistance and membrane potential ($r = 0.58$, $P < 0.001$); extrapolation of the linear regression line to a membrane potential of −80 mV suggests that the 'normal' input resistance is about 315 kΩ.

Response to nerve stimulation. Intracellular recordings of evoked and spontaneous synaptic potentials and currents were recorded from most biopsy samples. Typical examples of such recordings are shown in Fig. 4. The features of these synaptic events in the two groups of patients are described below and summarized in Table 7.

Endplate potentials. These could easily be recorded in most fibres in response to stimulation at 1 Hz. Often several penetrations were made at different places along the same fibre in an effort to ensure that the recordings were focal, as signalled by maximum amplitude and fastest time to peak. In spite of this the decay time constant (5.4 ms) was noticeably slower than in comparable preparations from mouse muscle (2.5 ms, using similar methods in our laboratory).

The mean EPP amplitude, normalized to a membrane potential of −80 mV, did not differ significantly between the two patient groups. The mean of all the patients was 10 mV. It should be noted that while we have made a correction for the effect of reduced membrane potential in these cut fibres, we were not able to make a correction for the decrease of input resistance resulting from damage. However, it can be seen from Table 7 that the sample with the lowest EPP amplitude is that with the largest mean fibre diameter

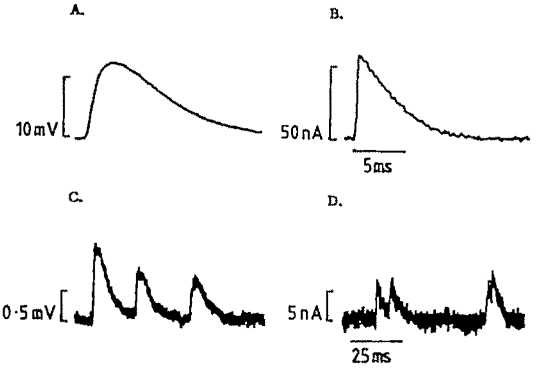

FIG. 4. Typical examples of synaptic potentials and currents all recorded from the same muscle fibre. Membrane potential −60 mV. A, endplate potential; B, miniature endplate potentials; C, endplate current; D, miniature endplate currents.

TABLE 7. ELECTROPHYSIOLOGICAL FEATURES OF NMJs IN *VL* STUDIED IN ISOLATED NERVE-MUSCLE PREPARATIONS

Patient no.	*EPP (mV)*	*EPC (nA)*	*H (mV)*	*mEPP (mV)*	*mEPC (nA)*	*mEPC*τ *(ms)*	*Mp/d*
Group 1							
17	10.1	77.7	105.2	0.52	5.28	3.94	22.2
30	7.4	48.8	160.5	0.66	4.69	3.74	15.0
37	10.5	62.0	167.2	0.53	3.69	4.06	22.5
38	9.9	49.3	134.2	0.66	4.31	3.74	17.2
39	13.7	50.3	118.2	0.90	4.55	3.27	20.3
Average	10.3	57.6	137.1	0.65	4.50	3.75	19.4
SD	2.3	12.5	26.6	0.15	0.58	0.30	3.2
n	5	5	5	5	5	5	5
Group 2							
13	4.7	65.8	97.3	0.32	–	–	20.7
15	11.4	60.5	–	0.85	–	–	21.7
16	15.2	–	–	1.03	–	–	31.1
31	7.0	69.0	150.0	0.56	5.76	2.80	19.7
Average	9.6	65.1	123.7	0.69	5.76	2.80	23.3
SD	4.6	4.3	37.3	0.31	–	–	5.3
n	4	3	2	4	1	1	4
F-test	ns	ns	ns	ns	–	–	ns
t test	ns	ns	ns	ns	–	–	ns
All							
Average	10.0	60.4	133.2	0.67	4.71	3.59	21.2
SD	3.3	10.4	27.3	0.22	0.73	0.47	4.5
n	9	8	7	9	6	6	9

(MPB no. 13), consistent with the expected relationship between fibre diameter, input resistance and EPP amplitude.

To assess the effect of nerve stimulation in a way that did not depend upon the variable effects of muscle fibre damage, and to obtain a more direct indication of the kinetic properties of the AChR associated ion channels at the NMJ, we recorded endplate currents.

At individual NMJs, the amplitude of the EPC varied linearly with membrane potential. The mean reversal potential −5.49 ± 5.55 mV did not differ significantly between the two patient groups and the pooled mean was not significantly different from 0 mV. The mean amplitude, estimated at a membrane potential of −80 mV, did not differ significantly between the two patient groups. The pooled mean was 61.8 nA.

Endplate currents in all patients had a rate of decay which was similar to that recently reported for human intercostal muscles (Engel *et al.*, 1990; but *see* Cull-Candy *et al.*, 1980) but significantly slower than for laboratory mammals (e.g. Vicini and Schuetze, 1985; Lyons and Slater, 1991). The constant, H, which characterizes the voltage dependency of the exponential decay time constant, has a value of about 140 mV, slightly greater than reported for intercostal muscles (Cull-Candy *et al.*, 1979). At −80 mV, the decay time constant was similar in the two groups of patients with a pooled mean of 4.1 ms.

Spontaneous transmitter release. The resting frequency of mEPPs and mEPCs was only a few per minute at most NMJs and was not determined accurately. At most NMJs the mEPP frequency increased greatly after high frequency stimulation, usually decaying to near rest values in less than 1 min. Miniature endplate potentials and mEPCs were recorded in samples from all patients in Group 1 but adequate numbers of mEPCs for analysis were only recorded in one sample from Group 2.

There was no significant difference in the amplitude or time course of mEPPs between the two groups of patients, and the features of mEPCs in the one patient from Group 2 were similar to those from Group 1. The mean amplitude of mEPCs from all the 47 NMJs studied (4.4 nA) was somewhat higher than that reported previously for human intercostals [2.6 nA, Cull-Candy *et al.* (1980); 3.8 nA, Engel *et al.* (1990)]. Miniature endplate potentials at fewer than 15% of NMJs in *VL* had values smaller than 2.6 nA (Fig. 5A). The mean exponential decay time constant of mEPCs was 3.7 ms, longer

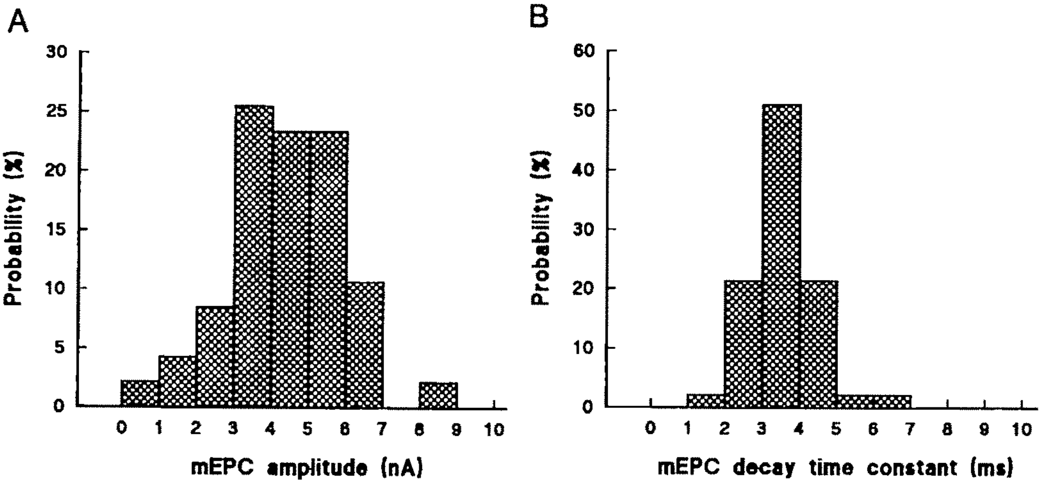

FIG. 5. Frequency distribution of the amplitude and exponential decay time constant of mEPCs recorded from NMJs in *VL*. Data from 47 NMJs in five patients in Group 1 and one patient in Group 2 are pooled.

than that reported by Cull-Candy *et al.* (1980, 1.7 ms) but similar to that reported by Engel *et al.* (1990, 3.25 ms).

Quantal content. The number of quanta released by a nerve impulse was estimated by both the 'direct' and 'variance' methods at 126 NMJs. The estimates of quantal content obtained after using the variance method were consistently greater than those obtained by the direct method (Fig. 6; mean for all nine patients, 37 ± 17 by variance, 21 ± 4 by direct method). Since the variance method depends on assumptions that are probably not true in solutions containing normal concentrations of calcium and magnesium (McLachlan, 1978), in what follows we have used only values determined by the direct method.

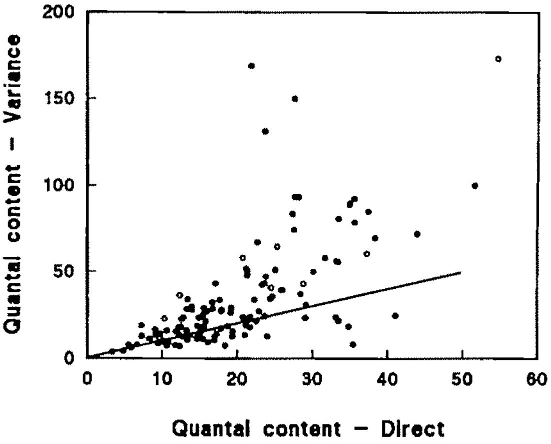

FIG. 6. Comparison of quantal content estimated by the 'direct' method with that estimated by the 'variance' method. The straight line shows equality of the two estimates. Note that at most NMJs, the variance method gives a substantially larger estimate. Closed circles = patients in Group 1; open circles = patients in Group 2.

The mean quantal content did not differ significantly between the two patient groups and had a pooled mean value of 21.1 (Table 7). At 1 Hz, the amplitude of the initial EPP is usually at least 25% greater than the mean value determined from a long series of responses. This suggests that at very low frequency, the quantal content may be 25–30.

At NMJs studied under voltage clamp, it was possible to estimate the quantal content using EPCs, making unnecessary any correction of the evoked response amplitude for non-linear summation (McLachlan, 1978). These estimates of quantal content, though based on a smaller sample than those using EPPs (46), were consistently smaller with a mean for all the NMJs studied of about 70% of that obtained with EPPs.

Statistics of transmitter release. In rat muscle, the value of the binomial parameter *n* estimated from the run down of successive responses in a high frequency train is an order of magnitude greater than that obtained from statistical analysis of trains at 1 Hz as described in Methods (Glavinović, 1979*b*).

We have obtained estimates of *n* and *p* using both methods from a number of fibres from patients in Group 1. Analysis of trains at 1 Hz gave a value of *p* of 0.59 while the 'run down' method gave a value of 0.05. The corresponding values of *n* in these fibres were 39 and 433, respectively.

DISCUSSION

Overview

The essential features of NMJs in the human *VL* are similar to those of NMJs in other human muscles which have been described by others (Engel, 1986). We are aware that, as in most other studies of human NMJs, none of the 'control' patients we studied was fully normal. We cannot, therefore, totally exclude the possibility that the condition which required the removal of a biopsy sample may have had some influence on motor innervation. However, our SFEMG recordings showed no signs of reorganized motor units. In addition, the mean value of jitter in patients in Group 1 was not significantly different from that in normal subjects. While some of the patients in Group 2 had greater than normal jitter, this is not necessarily indicative of disturbed neuromuscular transmission but may instead be related to variable conduction velocity along fibres with abnormally functioning membranes or due to prominent velocity recovery function effects (Stålberg and Trontelj, 1979).

We believe, therefore, that most of the features which we have described would be shared by those from individuals with no recognized medical abnormality. In this regard we are particularly struck by the fact that we have detected few if any consistent differences between NMJs in the patients in Group 1 and in those with overt myopathic conditions. In addition, many features of the structure and function of the NMJs we have studied are similar to those described by others in human intercostal muscles from control patients (e.g. Engel, 1986). While age related changes in the properties of human NMJs have been reported (e.g. Arizono *et al.*, 1984; Oda, 1984, 1985), we have seen no evidence of this, probably because both the size and age range of our sample was too small. We conclude that, taken together, these patients with no evidence of neurogenic involvement represent a useful group with which to compare patients with various forms of neurogenic disease.

While the NMJs we have described are qualitatively similar to those in muscles of mammals and lower vertebrates, there are some striking quantitative differences [*cf* Lyons and Slater (1991) for a similar analysis of a muscle in the mouse]. These include the relatively small size of the NMJ as a whole which is accentuated by the low occupancy of the synaptic region by the axon terminal and the low quantal content. On the other hand, the intensity of synaptic folding is greater in man than in lower vertebrates.

This pattern of features suggests that in the *VL*, and perhaps in human muscles in general when compared with those of lower vertebrates, the efficacy of neuromuscular transmission depends relatively more on the ability of the muscle fibre to generate an action potential in response to acetylcholine (ACh) released from the nerve than in the nerve's ability to release it.

The bulk of this discussion will concentrate on the process of transmitter release and the action of the transmitter on the postsynaptic membrane. It will end by considering, in a more speculative manner, the process of action potential generation in human muscle and its contribution to the efficacy of neuromuscular transmission.

Transmitter release

Estimate of quantal content. Previous studies of transmitter release at human NMJs have estimated the quantal content to be 40–250 (Elmqvist and Quastel, 1965; Cull-Candy *et al.*, 1980; Engel *et al.*, 1990), with most recent studies favouring values at the low end of this range. By contrast, we have found a value close to 20. In making this estimate, we have used the original correction for non-linear summation of quanta proposed by Martin (1955). For our material, this correction increases the estimate of quantal content by about 15%. There is reason to believe that this correction provides an upper limit to the true quantal content (Martin, 1976; Stevens, 1976). Following the reasoning in Martin (1976), and taking into account values we observed for passive cable time constant, EPC decay time constant and the amplitude of the EPP, it is likely that the true quantal content is only about 10% greater than the uncorrected value. We have continued to use the original correction primarily because of its ease of application.

In nearly all the earlier studies, quantal content was estimated in preparations in which transmitter action was partially blocked with d-tubocurarine (dTC). Since mEPPs are generally undetectable in blocking doses of dTC, quantal content was estimated using the 'variance' method. As is well known, and we have once again confirmed, use of the variance method systematically overestimates the quantal content determined by the 'direct'method (Fig. 6). Our estimate of quantal content, using the variance method (37), is very close to that reported with the same method by Engel *et al.* (1990) (41) in human intercostal muscles, suggesting that there is little real difference in transmitter release between these two muscles. In addition, there is evidence that dTC *per se* increases quantal content measured at 1 Hz through some action on the presynaptic nerve terminal (Ferry and Kelly, 1988).

One distinctive feature of our studies is that we have used cut fibre preparations to study neuromuscular transmission (Barstad, 1962). While it might be thought that damage to the muscle fibres would influence the transmission process, possibly by virtue of the leakage of K^+ from the cut fibres, there is evidence against this. Glavinović (1979*a*) has made a detailed study of transmitter release in cut fibre preparations from rat diaphragm. He concludes that in such preparations, the quantal content is relatively insensitive to the concentration of K^+ in the external medium and to temperature. In the conditions we have used, each factor might be expected to lead to a small (about 10%) reduction in quantal content. We therefore consider it unlikely that the relatively low values of quantal content we report result from the nature of our preparations.

The quantal content in human muscles seems, in general, to be lower than in the muscles of small mammals. In previous studies using cut fibre preparations, values of 30 – 130 have been reported in different muscles of the rat (Glavinović, 1979*a*; Di Gregorio *et al.*, 1990; Grubb *et al.*, 1991). In recent studies of the mouse epitrochleoanconeous muscle (ETA) (Bradley *et al.*, 1989), using methods identical to those used in the present study, we found a value of about 50. We therefore conclude that quantal content in human limb muscles is lower than in muscles from small laboratory animals in spite of the fact that the human muscle fibres are generally larger in diameter (about 50 μm vs 30 μm) and might be expected to have a higher quantal content to compensate for the presumed lower input resistance (Katz and Thesleff, 1957).

Structural correlates of transmitter release. In frogs, the quantal content at an individual NMJ is related to the size of the nerve terminal (Kuno *et al.*, 1971), implying that the release per unit area of synaptic contact is roughly constant. In the frog cutaneous pectoris muscle (CP) it seems that the total synaptic area is about 1200 μm^2 and the quantal content about 200 so that the release per unit area is about 0.17 quanta/μm^2 (data derived from Torri-Tarelli *et al.*, 1990). In NMJs of *VL*, the quantal content at 1 Hz corresponds to approximately 0.27 quanta/μm^2 (mean quantal content 21, mean synaptic area 79 μm^2). In the mouse ETA, where the quantal content is about 50, the release per unit area is similar (mean synaptic area 280 μm^2, 0.18 quanta/μm^2). Taken together, these data (Table 8) imply that an important factor determining the relatively low quantal content at human NMJs is their small size.

Transmitter release occurs by exocytosis of transmitter-containing vesicles at sites known as active zones (e.g. Heuser *et al.*, 1979). The density of these active zones in the nerve terminals of man and mice is about 2.6/μm^2 (Fukunaga *et al.*, 1982, 1983; Fukuoka *et al.*, 1987). In *VL*, where about 0.27 quanta are released per μm^2, this implies the release of about 0.1 quantum of transmitter/active zone for each nerve impulse at 1 Hz.

TABLE 8 COMPARISON OF NMJs IN FROG, MOUSE AND MAN

Species	*PreA* (μm^2)	*M*	*M/PreA*	*PostA* (μm^2)	*PostA/PreA*
Frog CP (1)	1200	200	0.17	3000	2.50
Mouse ETA (2)	280	50	0.18	2012	7.20
Human *VL* (3)	79	21	0.27	1616	20.50

(1) Data as cited in Torri-Tarelli *et al.* (1990) and derived from a variety of published micrographs; (2) data from S. A. Bradley, P. R. Lyons and C. R. Slater (unpublished observations); (3) this study.

The declining response to repetitive stimulation at high frequency, possibly a very important feature of the normal function of human NMJs, has been interpreted in terms of a pool of 'immediately available' quanta (Elmqvist and Quastel, 1965). Recent studies by Engel *et al.* (1990) suggest that at NMJs of human intercostal muscles there are 300–400 such quanta. Our own observations suggest that in *VL*, the number is similar. In *VL*, the total number of active zones per NMJ is approximately 200 (2.6 active zones/μm^2, 80 μm^2 total synaptic area). It thus seems that the number of readily releasable quanta, as inferred from the decline in quantal content during stimulation at 20–50 Hz, is of the order of two per active zone.

Both our studies and those of others (Glavinović, 1979*b*; Grubb *et al.*, 1991) suggest that the number of 'immediately available' quanta determined from run-down studies is much greater than the value of the binomial variable n as calculated from the variation of quantal content in successive responses at 1 Hz, as assumed by others (Elmqvist and Quastel, 1965; Engel *et al.*, 1990). At present it remains unclear whether the variables n and p of the mathematical description of transmitter release as a binomial process refer to any discrete process within the axon terminal, such as depletion of one particular vesicle store, as opposed to a complex combination of as yet poorly defined events.

Transmitter action

Our observations indicate that the decay of synaptic currents is slower at NMJs in human *VL* (τ_{mEPC} 3.6 ms) than at NMJs in the small laboratory mammals that have been studied (τ_{mEPC}1–2 ms; e.g. Quastel and Linder, 1975; Vicini and Schuetze, 1985). In an early report, the value of τ_{mEPC} at NMJs in human intercostal muscle was reported as 1.7 ms (Cull-Candy *et al.*, 1979). More recently, Engel *et al.* (1990) have found values very similar to ours. The recorded rate of decay of mEPCs and EPCs is dependent on the proximity of the recording and current passing electrodes to the NMJ (Quastel and Linder, 1975; Cull-Candy *et al.*, 1979). We think it is unlikely that imprecise electrode placement explains our results for two reasons. First, because we made efforts to ensure that our electrodes were placed so as to record responses with maximum amplitude and minimum decay time. Secondly, because only a very small fraction of the total population of mEPC and EPC decay times we observed were shorter than 2 ms (*see* Fig. 5B). We therefore conclude that the synaptic currents are truly more prolonged at NMJs in *VL* than has been reported for NMJs in small laboratory animals.

In most vertebrate muscles, τ_{mEPC} is similar to the mean open time of the AChR channels that generate it. In adult bovine muscle the mean channel open time, determined both from single channel recording and estimated from mEPC decay time constant (5.6 ms; Mishina *et al.*, 1986) is considerably longer than in most small laboratory animals where it is normally 1 – 1.5 ms [e.g. Vicini and Scheutze (1985), rat]. These differences in ACh-gated ion channel properties presumably reflect the diversity in primary structure of the various subunits of the AChR molecule in different species.

The rate of decay of synaptic currents has an important functional consequence. For a synaptic current with a rapid rise and a simple exponential decay, the total charge movement across the membrane, and hence the peak depolarization, is related to the rate of decay of the current transient. In other words, a slowly closing channel allows greater depolarization of the muscle fibre per ACh molecule than a rapidly closing channel with the same conductance. In large animals, such as man, the relative lack of need for very high frequency activation of muscle may allow an economy in terms of the amount of transmitter release.

Action potential generation in human muscle

The generation of an action potential in the muscle fibre is the essential outcome of neuromuscular transmission. In most studies of this process, the electrical threshold for action potential generation has been determined in the extrajunctional region where it is normally –55 mV to –60 mV. On the basis of our observations in *VL*, it seems that during stimulation at 1 Hz, even allowing for some effect of muscle fibre damage on input resistance, the EPC is only just large enough to bring the membrane potential to this level. Single fibre EMG studies, including our own, indicate that there is no failure of neuromuscular transmission in *VL* in normal conditions. This suggests a significant discrepancy between performance *in vivo* and what we record *in vitro*.

One possible explanation for this apparent discrepancy is that the minimum depolarization required to generate an action potential may be smaller *in vivo* than in isolated muscles. McComas *et al.* (1968) made intracellular recordings from quadriceps muscle in patients and found the 'critical membrane depolarization' to be only about 12 mV, starting from a resting potential of about –84 mV. This implies an action potential threshold of –72 mV which is considerably more negative than in muscles studied *in vitro*. An additional factor may be the temperature at which we have made our recordings. The study of Glavinović (1979*a*) on rat muscle indicates that quantal content increases about 1.35-fold as the temperature is raised from 22 °C to 37 °C. This implies a quantal content in *VL* at 37 °C of about 28.

A further explanation for this apparent discrepancy may be that the action potential threshold at the NMJ is more negative than in the extrajunctional region of the muscle fibre, where it is usually recorded. For some years, electrophysiological evidence has been accumulating which indicates the presence of a high density of voltage-dependent Na^+ channels at the vertebrate NMJ (e.g. Caldwell *et al.*, 1986). Recently, these findings have been complemented by immunocytochemical studies indicating a very high concentration of Na^+ channels at the NMJ (Angelides, 1986; Flucher and Daniels, 1989). The study of Flucher and Daniels (1989) showed elegantly that the Na^+ channels are present in highest concentration in the depths of the synaptic folds, where their concentration appears to be 10–100 times greater than in the extrajunctional region. The presence of a high density of channels means that a smaller fraction of the channels needs to be open to give rise to a sufficient current to trigger an action potential. As a result, it is likely that the threshold voltage for action potential generation is indeed lower at the NMJ than elsewhere.

It is possible to estimate the magnitude of the effect that such an accumulation of Na^+ channels might have at NMJs in *VL*. Since the space constant of decay of the EPP is several hundred of micrometers, most of the Na^+ channels at the NMJ would probably be acted on by an EPP, whether or not they were in immediate contact with the nerve. If, as seems likely, the absolute density of these channels in the postsynaptic membrane is of the order of $10^3/\mu m^2$ (Flucher and Daniels, 1989) then, recalling that the area likely to be occupied by a high density of Na^+ channels is about 650 μm^2 (*see above*), the total number at an average NMJ in *VL* is of the order of 6.5×10^5/NMJ. If each Na^+ channel has a conductance of 18 pS (Hille, 1984), the total conductance arising from opening all of these channels at a single NMJ would be about 1.2×10^7 pS. For comparison, the total conductance change associated with the EPCs we have recorded is about 1×10^6 pS. It thus seems that the large number of voltage-dependent Na^+ channels at the human NMJ may act as a potent amplifier of the EPC, ensuring effective activation of the muscle fibre in spite of the relatively low quantal content.

It is likely that the relative effect of the junctional accumulation of Na^+ channels is different in different species. In Table 8 we compare a number of features of NMJs in frog, mouse and man. In all three species, the quantal content per unit area of axon terminal is roughly the same (*see above*). By contrast, other features of the NMJ are strikingly different. In the frog, although the muscle fibres are only slightly larger than in the *VL* of man, the area of presynaptic contact and the quantal content are both 10–15 times greater. The intensity of folding, however, is much less in frog than in man, with the result that the ratio of the total area of postsynaptic membrane to the effective presynaptic area is nearly 10 times greater in man than in frog, while mouse NMJs are intermediate. It thus seems likely that any current-amplifying effect would be significantly greater in NMJs of man than in those of frog or mouse.

Conclusions

We were initially puzzled by our finding that the NMJs in human *VL* are relatively small and characterized by low quantal content. One explanation for these findings may be the existence of a spectrum of the structure-function relationship of vertebrate NMJs. At one end may be those such as in frog, characterized by a large presynaptic component and high quantal content but relatively little synaptic folding. In these, the safety factor of transmission may be largely determined by the amount of transmitter released. Human NMJs may be near the other end, characterized by a relatively small presynaptic component and low quantal content, but extensive folding associated with a large number of voltage-dependent Na^+ channels. Here, the effect of the transmitter may be significantly enhanced by postsynaptic amplification.

If such a view is correct, then any pathological condition in man which leads to a decrease in the extent of folding at the NMJ, such as described by Smit *et al.* (1988), might of itself have a deleterious effect on neuromuscular transmission.

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RESPIRATORY INVOLVEMENT IN MULTIPLE SCLEROSIS

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SUMMARY

Respiratory complications occur in advanced multiple sclerosis (MS) but may also complicate acute relapses earlier in the disease. We present 19 patients with MS who developed respiratory complications at a mean of 5.9 (range 1–12) yrs after the onset of neurological symptoms.

Fourteen patients developed severe respiratory insufficiency presenting with a combination of reduced forced vital capacity (FVC), hypoxaemia or hypercapnia (12 patients) and respiratory arrest (four patients). Two patients presented with apneustic breathing, one with paroxysmal hyperventilation, one with obstructive sleep apnoea and one with bulbar weakness leading to aspiration pneumonia. Respiratory muscle weakness was a major factor in 14 patients (predominantly diaphragm involvement in six), bulbar weakness in seven patients, impaired voluntary control in three and impaired automatic control in three.

Twelve patients received mechanical respiratory support of whom seven have subsequently died. The methods of support used were intermittent positive pressure ventilation (nine patients), iron lung (three), cuirass (two) and rocking bed (one). Six patients were maintained on respiratory support until they died after intervals varying from 24 h to 6 yrs (mean 17.7 mths). Five patients received temporary ventilation for between 6 d and 42 d: of these four remain alive at up to 4 yrs and one died after 16 yrs. One patient remains on domiciliary nasal intermittent positive pressure ventilation (IPPV) after 1 yr.

INTRODUCTION

Respiratory complications are common in the terminal stages of multiple sclerosis (MS) and death is often due to bulbar weakness leading to aspiration or bronchopneumonia (McAlpine and Compston, 1952; Kurtzke, 1970). However, respiratory involvement may occur earlier in the course of the disease, particularly during relapses (Guthrie *et al.*, 1952).

Whilst some authors suggest that acute respiratory failure is rare in MS and usually reflects extensive bulbar disease (Hallpike *et al.*, 1983; Matthews *et al.*, 1985), others state that acute, occasionally fatal, respiratory failure is not uncommon (Kurtzke *et al.*, 1970). Respiratory insufficiency has been described in occasional patients (Guthrie *et al.*, 1952; O'Donohue *et al.*, 1976; Douglas *et al.*, 1983). However, the detailed analysis of acute respiratory involvement in MS is often complicated by secondary systemic factors such as dehydration, aspiration pneumonitis and sepsis (Boor *et al.*, 1977).

We present a series of 19 patients which illustrates the wide range of respiratory disorders seen in MS.

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METHODS

Nineteen patients with major respiratory symptoms associated with MS were seen in the medical intensive care unit at the National Hospital for Neurology and Neurosurgery and the Lane Fox Respiratory Unit, St Thomas' Hospital between 1971 and 1990. Clinical details were obtained either by direct observation or retrospectively from case notes. Assessments included careful evaluation of the criteria used to diagnose MS, the features of clinical presentation and the development of respiratory symptoms and signs. Only clinically or laboratory supported definite cases (Poser *et al.*, 1983; McDonald and Silberberg, 1986) or cases in which the diagnosis was confirmed at post-mortem were included. Note was taken of respiratory investigations, the nature of ventilatory support and any symptomatic benefit Where possible the patients were recalled for review or, if deceased, the general practitioner was contacted and full details obtained. The subsequent disease progression and outcome was noted. Autopsy findings were reviewed in three cases.

Arterial blood gas samples had been analysed immediately on an ABL2 blood gas analyser (Radiometer Ltd, UK). Nocturnal oximetry was performed in eight patients (Cases 7–9, 10, 12, 13, 14, 16) and respiratory muscle strength was assessed in two patients (Cases 9, 14). Respiratory failure was defined as PaO_2 less than 8 kPa (60 mmHg) alone or with $PaCO_2$ greater than 6.7 kPa (50 mmHg) (Sykes *et al.*, 1976).

One case in this series has been previously reported (Cooper *et al.*, 1985).

RESULTS

There were six males and 13 females, three of the females were of West Indian origin, one female was from India and one male from Pakistan. The time between the onset of MS and respiratory involvement was 5.9 yrs (±4.8 yrs; range 1–12 yrs). No patients had respiratory symptoms or signs at the onset of MS. In June 1990 eight patients were under regular review, nine were known to have died and two patients had been lost to follow-up.

The MS had an initially relapsing and remitting course in 17 patients and was progressive without remissions in two. Details of clinical involvement are shown in Table 1 and described in the text. Sixteen patients had received multiple courses of steroids and five had received cyclophosphamide and azathioprine.

The patients are considered below according to the major causes of respiratory impairment found, i.e. respiratory muscle weakness, bulbar dysfunction, obstructive sleep apnoea, abnormalities of respiratory control and paroxysmal hyperventilation. In several patients more than one cause was found (Cases 1–14).

Respiratory muscle weakness (Table 2)

Fourteen patients developed severe respiratory difficulties manifest as dyspnoea, orthopnoea or confusion and obtundation. Ten had overt respiratory failure and of these two had a respiratory arrest. Clinical signs of diaphragmatic weakness were present in six patients (Cases 9–14). One patient (Case 1) was severely disabled by previous poliomyelitis and in another patient (Case 6) there was a past history of myasthenia gravis in full clinical remission following thymectomy. Of these 14 patients, six presented with acute onset of respiratory symptoms (less than 24 h) and eight with subacute onset (>24 h) (Table 2). Three patients (Cases 3–5) presented with acute confusion, drowsiness and obtundation, two patients with progressively increasing dyspnoea and two patients had a respiratory arrest. Three patients had poor cough and frequent choking and one patient had no specific respiratory symptoms (Case 7). On examination the confusion and obtundation were noted in three patients. There was reduced chest wall movement and air entry in all the patients. Four had lower respiratory tract infections

TABLE 1 PRESENTATION AND SITES OF LESIONS (DETERMINED CLINICALLY AND BY MRI, POST-MORTEM OR BOTH)

Case	*Age at onset of respiratory symptoms (yr)*	*Sex*	*Year*	*Duration after onset of MS (yr)*	*MRI*	*PM*	*Optic nerve*	*Hemisphere*	*Brainstem*	*Spinal cord, cervical, thoracic (level represents upper border)*
1	51	F	1981	10					+	+
2	36	M	1978	18			+		+	+
3	58	F	1976	8					+	+
4	21	F	1973	1		+	+		+	+ (C2)
5	47	F	1972	1			+			+
6	50	F	1989	5	+	+	+	+	+	+ + (T6)
7	19	F	1990	3	+		+			+ (C5)
8	62	F	1990	2	+					+ (C2)
9	28	F	1989	7	+		+	+	+	+ (C2)
10	38	F	1986	1	+		+	+	+	+ (C2)
11	41	M	1987	3	+			+		+ (C4)
12	27	F	1979	5		+	+	+		+ (C2)
13	25	F	1988	2	+		+	+	+	+ (C2)
14	41	F	1984	3			+			+ (C2)
15	17	F	1989	4	+		+	+	+	+ (C2) + (T4)
16	23	M	1984	2	+		+	+	+	+ (T10)
17	35	M	1971	7			+		+	+ (T4)
18	51	F	1974	9						+ (C5) + (T1)
19	28	F	1974	1			+		+	+

TABLE 2 PRESENTATION AND CAUSE OF RESPIRATORY INVOLVEMENT

	Onset of respiratory insufficiency						*VC (litres)*			
Case	*Acute <24 h*	*Subacute >24 h*	*Respiratory failure*	*Respiratory arrest*	*Generalized respiratory muscle weakness*	*Diaphragm weakness*	*Erect*	*Supine*	*Bulbar*	*Abnormal breathing pattern*
1		+	+		+		0.6 (2.6)		+	
2		+	+		+		0.7 (4.5)	0.6	+	+ (loss of voluntary control)
3	+		+	+	+		(TV 0.2)		+	
4	+		+		+		(TV 0.3)			+ (loss of voluntary control)
5	+		+		+					+ (loss of automatic and voluntary breathing)
6		+	+	+	+			0.7	+	+ (paroxysmal hyperventilation)
7		+			+		1.6 (4.3)	1.3		
8	+		+	+	+		0.5 (3.1)			
9		+			+	+	3.1 (4.2)	0.8	+	
10		+			+	+	1.8 (3.8)	0.8		
11	+		+		+	+	0.9 (4.2)	0.6		
12	+		+		+	+	2.5 (3.0)	0.9		+ (apneustic/ ratchet breathing)
13		+	+		+	+	0.5 (4.0)	0.2		
14		+			+	+	2.0 (4.6)	0.9		
15	+		+				1.7 (4.1)	1.3	+	
16		+							+	+ (obstructive sleep apnoea)
17	+						3.8 (4.5)		+	+ (apneustic breathing)
18		+					3.4 (4.5)			+ (apneustic breathing)
19		+					2.7 (3.8)		+	+ (paroxysmal hyperventilation)

and two of these were cyanosed. There was also impaired voluntary control of respiration in three patients (*see below*). There was obvious paradoxical movement of diaphragm in six patients.

Forced vital capacity (FVC) measurement was available in 11 patients, three others were unable to perform the manoeuvre but tidal volume (TV) was assessed in two of these. In all the patients FVC was reduced substantially below the predicted value and in six patients the FVC was below 1 l or TV below 300 ml. In six patients (Cases 9–14) FVC fell by a third or more from sitting to supine (Table 2). Inspiratory mouth occlusion pressures and transdiaphragmatic pressure measurements were performed in two patients and confirmed global respiratory muscle weakness with predominant diaphragmatic weakness. Chest X-ray confirmed lower lobe consolidation in three patients. Arterial blood gases showed hypoxaemic respiratory failure in eight patients and hypoxic hypercapnic respiratory failure in four patients; they were normal in one patient and not recorded prior to intubation and ventilation in another.

At the time of respiratory symptoms neurological involvement was always extensive in these 14 patients. Ten patients had optic atrophy and 10 had eye-movement abnormalities. There were obvious bulbar signs in five (Cases 1–3, 6); these included dysarthria, reduced pharyngeal reflexes and palatal movements, paralysed vocal cords and slowed tongue movements. Eight patients were either totally quadriplegic or had spastic paraplegia with severe upper limb weakness, five patients had spastic paraplegia with moderate upper limb weakness and one had only mild upper limb weakness. Sensory involvement included high cervical sensory levels to all modalities (10 patients), a thoracic level (one) and patchy variable loss (three). In all the patients with reduced voluntary control of breathing there was loss of dorsal column sensation.

Seven patients had one or more MRI scans and typical periventricular and hemispheric white matter lesions were seen in six. In five patients (Cases 6, 9–11, 13) there was high signal on T_2-weighted images or atrophy of varying extent from the lower medulla into the upper thoracic cord and in three there was extensive abnormal signal in the cervical cord (Cases 6–8) (Figs. 1, 2).

Three cases subsequently underwent autopsy. In the first patient (Case 4) there was loss of voluntary respiratory control and respiratory muscle weakness. Death was due to bronchopneumonia. There were numerous discrete plaques in the hemispheric white matter. The lower mesencephalon showed recently formed irregular plaques in the periaqueductal grey matter, with extension into the medial lemniscus on one side. At the mid-pontine level there was extensive involvement by plaques of recent origin scattered throughout the tegmentum. Plaques were also noted to be in the dorsal portions of the superior cerebellar peduncles and to involve the mesencephalic root and nucleus of the trigeminal nerve, medial longitudinal fasciculus (MLF) and superior medullary velum. In the caudal pons (Fig. 3) there was extensive tissue involvement including MLF, medial lemniscus, trapezoid body, fifth, sixth and seventh nerve nuclei, transverse pontine fibres and long tracts. At the pontomedullary junction and in the medulla (Fig. 4) plaques were confined to the floor of the fourth ventricle and dorsolateral regions with involvement of the eighth nerve, especially the vestibular nuclei, and the nucleus tractus solitarius. Sections taken through abnormal areas in the mid-cervical and mid-thoracic spinal cord showed extensive demyelination involving the ventral grey matter, with only a few patchy, asymmetrical areas of preservation.

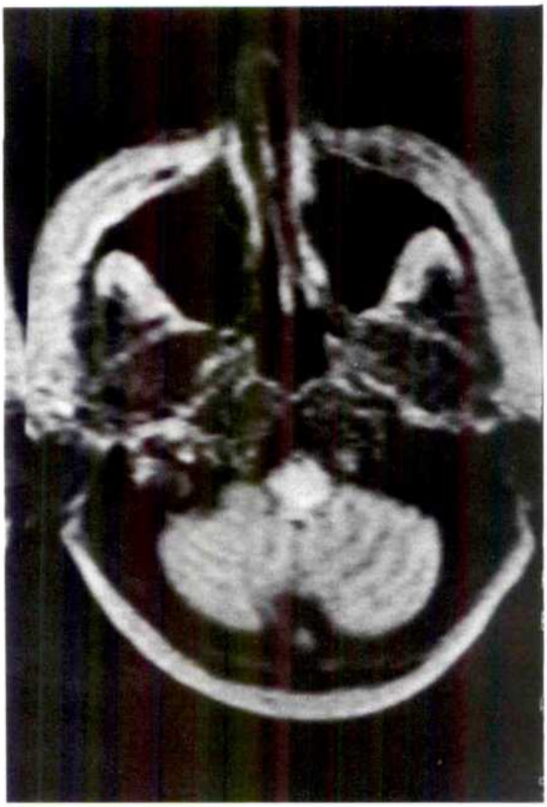

FIG. 1. Unenhanced T_2-weighted axial MRI showing high signal in the medulla (*Case 6*).

In the second patient (Case 6) there was respiratory muscle and bulbar weakness and episodes of paroxysmal hyperventilation. Death was from bronchopneumonia. Macroscopic examination showed lesions in the white matter, involving the top end of the left internal capsule at the level of the anterior commissure, the superior frontal gyrus; one in the second temporal gyrus, one in the right hemisphere and one in the left globus pallidus. The dorsal medulla was blurred with the tissues appearing grey and shiny and the spinal cord was thin and irregular. Histological examination showed sharply demarcated areas of acute myelin loss in the cerebral hemispheres and brainstem. Lesions in the medulla had sharp but irregular outlines involving both pyramids, medial lemniscus, MLF and both inferior olives. The cervical spinal cord was involved by an extensive and irregular loss of myelin and decreased numbers of axons. At the thoracic and lumbar level, the spinal cord was involved by extensive irregular loss of myelin and decreased numbers of axons.

The third patient (Case 12), who had clinical evidence of impaired automatic respiration and respiratory muscle weakness, died of bronchopneumonia. Recent demyelinating lesions were noted in the cerebral white matter involving the genu of the corpus callosum, right postero-frontal and parieto-occipital regions. There was an area of demyelination in the right middle cerebellar peduncle. The cervical and thoracic spinal cord and the cervical and most thoracic anterior roots were severely shrunken and grey.

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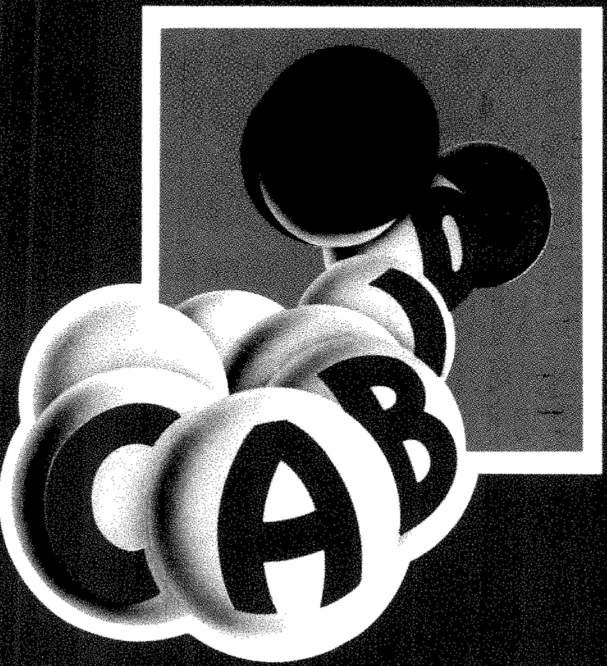

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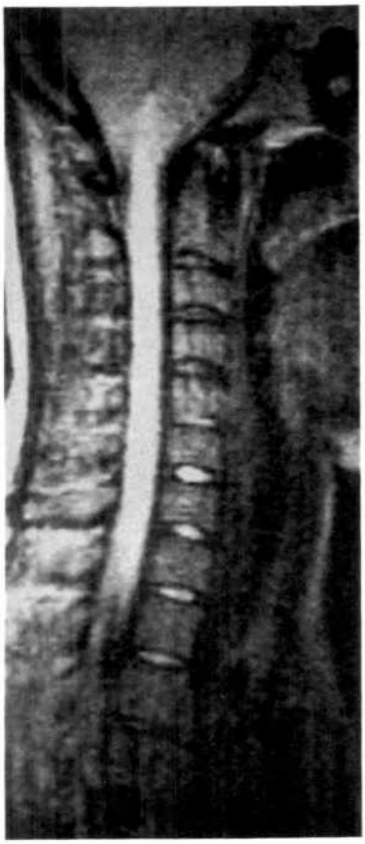

FIG. 2. Unenhanced T_2-weighted sagittal MRI of cervical cord showing extensive high signal lesion (*Case 7*).

Of the 14 patients with dominant respiratory muscle weakness eleven (Cases 1 – 3, 5, 6, 8, 9, 11 – 14) received mechanical support, five of whom also had a tracheostomy (Table 3). Six of these 11 required support until they died at times varying from 24 h to 6 yrs. The remaining five patients were weaned from a few days to 1 yr after the onset of support and have survived 1 – 16 yrs. Three of 14 patients did not receive mechanical support. One of them died and two substantially improved.

Bulbar dysfunction (Cases 1 – 3, 6, 9, 15 – 17, 19)

Bulbar dysfunction was present in nine patients. One patient (Case 15), with a previously relapsing and remitting acute cervical transverse myelopathy, presented with an acute bulbar relapse with vertigo, nausea, diplopia, dysarthria and dysphagia. She became progressively dyspnoeic with a productive cough. On examination there was impaired swallow and cough, bilaterally absent gag reflexes and slow tongue movements. She had developed extensive pulmonary consolidation in both lower zones and was considered to have aspiration pneumonia secondary to the bulbar relapse. An MRI scan several months prior to the acute episode had shown disseminated cerebral hemispheric and cerebellar lesions and an acute inflammatory lesion with swelling of the cervical cord.

On admission she was hypoxaemic with a supine vital capacity of 1.3 l. Chest X-ray

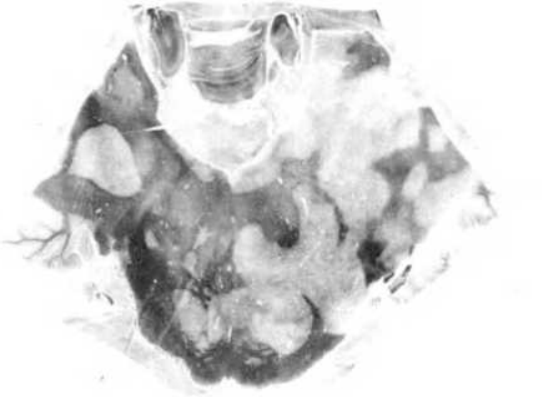

FIG. 3. Celloidin section of lower pons in *Case 4* (Loyez stain).

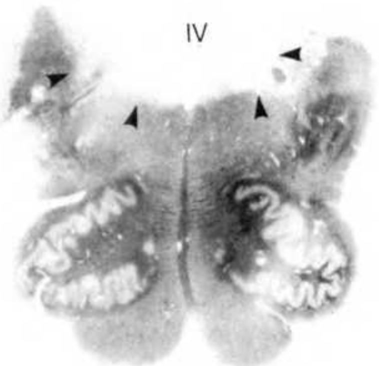

FIG. 4. Celloidin section of medulla in *Case 4* (Loyez stain). Arrows indicate plaques of demyelination. The fourth ventricle is indicated.

confirmed residual shadowing in the right upper and left lower zones. She was intubated and ventilated initially with IPPV but subsequently with negative pressure ventilation with an iron lung. She recovered from this relapse and ventilation was discontinued after 1 mth. She has suffered several subsequent relapses without respiratory involvement and remains alive after 1 yr.

The other eight patients had features which included loss of pharyngeal sensation and sensation of taste over the posterior third of the tongue (two patients), impaired speech and swallowing (six), palatal weakness or paralysis with reduced or absent gag reflex (six) and hiccuping (two). In six cases there was also clinical and radiological evidence of pulmonary consolidation thought likely to be due to aspiration and/or impaired sputum clearance. All these patients also had respiratory muscle weakness and/or disorders of the control of respiration.

Obstructive sleep apnoea (Case 16)

A patient with relapsing and remitting dysasthaesiae complained that towards the end of pregnancy her husband had noted that she snored and breathed irregularly at

TABLE 3. TREATMENT AND OUTCOME

Case	*Ventilated/ tracheostomy (type of tube)*	*Mode of support*	*Duration*	*Weaned from ventilatory support*	*Outcome*
1	+	Intermittent iron lung	1 yr	0	Died (1 yr) of bronchopneumonia
2	+	Intermittent cuirass	6 yrs	0	Died (6 yrs) of bronchopneumonia
3	+	Continuous IPPV	24 h	0	Died (24 h) of bronchopneumonia
4	0				Died (3 wks) of bronchopneumonia
5	+	Continuous IPPV	10 d	+	Died (16 yrs) of an accident
6	+/+ (cuffed)	Intermittent IPPV	1 mth	0	Died (1 mth) of bronchopneumonia
7	0				Alive (6 mths)
8	+/+ (cuffed)	Continuous IPPV	1 mth	+	Alive (2 mths)
9	+/+ (silver)	Nocturnal IPPV	1 yr	+	Alive (1 yr)
10	0				Alive (5 yrs)
11	+/+ (silver)	Continuous IPPV	9 mths	0	Died (9 mths) of pulmonary embolus
12	+	Intermittent cuirass and iron lung	1 yr 6 mths	0	Died (10 mths) of bronchopneumonia
13	+	Continuous IPPV	6 d	+	Alive (18 mths)
14	+/+ (cuffed)	Intermittent rocking bed and IPPV	2 wks 4 wks	+	Alive (4 yrs)
15	+	Continuous IPPV and iron lung	1 mth	+	Alive (1 yr)
16	0				Alive (2 yrs)
17	0				Lost to follow-up (2 yrs)
18	0				Died (14 yrs) of Ca bronchus
19	0				Lost to follow-up (1 yr)

night and occasionally stopped. She had a relapse 2 yrs later with palatal weakness, reduced pharyngeal reflexes and slow tongue movements. During this episode she had frequent nightmares and woke with frontal headache, fatigue and nasal stuffiness and her husband noted she had irregular breathing at night with frequent snoring and apnoeic periods lasting 5−10 s. Sleep studies showed episodes of desaturation to $SaO_2 < 70\%$ lasting approximately 4−5 min and associated with typical obstructive sleep patterns and bradycardia. Ear, nose and throat examination showed large inferior nasal turbinates and large tonsils. Following gradual remission of the neurological status she underwent tonsillectomy and trimming of inferior turbinates. There have been no further respiratory symptoms.

Abnormalities of respiratory control (Cases 2, 4, 5, 12, 17, 18)

Abnormal patterns of respiration, which could not be explained entirely on the basis of respiratory muscle weakness, were observed in six patients. One patient (Case 17) with relapsing and remitting brainstem and spinal-cord episodes was admitted because of apneustic breathing. He was noted to have paroxysms of gasping inspiration, culminating in apnoea in full inspiration and the gradual development of cyanosis before shallow and rapid respiration commenced. Within a few seconds he developed hyperventilation and returned to normal saturation. Apneustic breathing seemed to be triggered by taking a deep breath, emotional upset, swallowing or physiotherapy. Attacks did not occur during sleep and CO_2 rebreathing via a closed circuit appeared to abolish them. This pattern of inspiratory apneusis disappeared after 36 h of treatment with ACTH. There were four further relapses over the subsequent 18 mths in which apneustic respiration occurred. On each occasion this consisted of deep, prolonged inspiration, with an inability to expire, lasting up to 40 s; they were succeeded by over inspiration and then rapid gasping hyperventilation before returning to a more normal respiratory rate and rhythm. Furthermore, his wife had noted that he suffered prolonged periods of nocturnal apnoea, lasting up to 1 min. He continued to suffer episodes of apneustic breathing associated with relapses but subsequently was lost to further follow-up.

A second patient (Case 18) with a progressive spastic tetraparesis developed apneustic breathing. He complained of intermittent breathing difficulty with a feeling of constriction around his throat. He had frequent episodes of inspiratory apneusis with breathing ceasing at peak inspiration for approximately 30 s. Between the attacks he was able to cough and take a deep breath normally. He was treated with an O_2 enriched inspiratory gas mixture to protect him from the ventilatory effects of the spasms. The abnormal breathing patterns resolved over 2 mths but his progressive disability worsened until he was virtually quadriplegic. He remained in this state without any further respiratory involvement until the time of his death from carcinoma of the bronchus, 14 yrs later. Both these patients were able to alter their respiratory pattern voluntarily and perform an FVC manoeuvre except during the periods of apneusis.

Two patients, with respiratory muscle weakness, developed highly abnormal respiratory patterns without evidence of intrinsic pulmonary disease (Cases 5, 12). In the first patient (Case 12) there was clinical evidence of an anterior spinal-cord lesion at C2; the tidal volume and frequency were irregular, she found increasing difficulty breathing at night and frequently awoke with palpitations. Initially she was more comfortable and slept better in a noctural cuirass but her disease was progressive and she subsequently died from bronchopneumonia. This patient was able to control her ventilation voluntarily. The second patient (Case 5) had a spastic tetraparesis and was admitted unconscious in respiratory failure. After initial resuscitation she was noted to have ratchet breathing with apneustic pauses. Subsequently it became evident that she could not voluntarily increase her tidal volume or stop breathing. There was no evidence of intrinsic pulmonary disease. Voluntary control returned with remission of the acute relapse.

Two other patients were unable voluntarily to influence their pattern of respiration. Both patients had severe respiratory muscle weakness due to lesions of the corticospinal tracts in the cervical cord and have been described above. The first patient (Case 2) with a 10 yr history of MS had a severe quadriparesis, poor cough and reduced pharyngeal reflex. He was noted to have a regular respiratory rate with occasional intermittent involuntary deep sighs and was unable to voluntarily vary his own respiration to command, e.g. attempted breath holding, deep breathing or coughing. He was admitted in hypoxaemic respiratory failure but without evidence of intrinsic pulmonary disease. Clinically there was considerable respiratory muscle weakness and the diaphragm appeared to be the main effective respiratory muscle. The impaired voluntary control of respiration persisted, although a cuirass improved his ventilation. He continued intermittent domiciliary respiratory support until his death from bronchopneumonia.

The second patient (Case 14) had a rapid course with severe relapses involving optic nerves, hemisphère, brainstem and spinal cord. She was admitted rousable but drowsy with a further relapse involving the brainstem and upper cervical cord. She was observed to have a regular and unvarying pattern of breathing and was unable to perform a vital capacity manoeuvre, to hyperventilate, hold her breath or cough to command, despite being able to obey other commands. She subsequently developed prolonged apnoeic episodes lasting up to 60–70 s before developing bronchopneumonia from which she subsequently died.

Paroxysmal hyperventilation (Cases 2, 6, 19)

Two patients (Cases 19, 6) were noted to have episodes of paroxysmal hyperventilation. The first patient (Case 19) had an acute brainstem relapse following which she had a focal fit with left facial twitching and subsequently lost consciousness for 15 min. Over the next 3 wks she developed paroxysmal episodes which occurred up to seven times a day. The attacks were precipitated by sitting down and standing up or exercise. She became aware of the right side of her face being pulled up with a feeling of difficulty catching her breath and of her tongue being forced into the back of her throat. This was followed by rapid panting for 1 min. She never lost consciousness and was fully aware of her surroundings. During the attack she was noted to have contractions of the right side of the face followed by curling of the fingers of the right hand. This was followed by rapid, even respiration for about 1 min interrupted by apnoeic periods lasting approximately 20 s. These attacks resolved with remission of the relapse but the patient has been subsequently lost to follow-up.

The second patient (Case 6) was severely disabled by progressive bulbar and spinal-cord demyelination. She developed episodes of hyperventilation followed by irregular respiratory pattern with prolonged apnoeic periods. She remained unresponsive for approximately 10 min with slight fluttering of the eyelids and movement of the head.

A third patient (Case 2) developed episodes of distressing flexion spasms affecting her arms. These were precipitated by a brief period of hyperventilation and Chvostek sign was always negative. These episodes were thought to be a paroxysmal brainstem manifestation of demyelination triggered by hyperventilation.

DISCUSSION

The incidence of respiratory involvement in MS is uncertain. In the series of McAlpine and Compston (1952) the cause of death was known in 33 of 85 patients. There were three deaths from respiratory failure, usually in patients with acute disease who died within a few months of onset, one from status epilepticus and 14 from respiratory infections. Kurtzke (1970) describes 12 fatal instances of respiratory failure in MS or acute disseminated encephalomyelitis and even more cases which were not fatal. Respiratory failure was almost always due to paralysis or weakness of the respiratory muscles and generally occurred in the setting of rapidly developing quadriparesis or bilateral arm weakness alone. O'Donohue *et al.* (1976) described two cases of MS admitted to a respiratory intensive care unit because of respiratory insufficiency, one patient required intubation and ventilation but both patients survived the episode. However, in the series of Douglas *et al.* (1983) four patients with brainstem relapses of MS required ventilation. Two of these patients died. The causes of death were staphylococcal septicaemia and irreversible brain damage sustained during a cardiorespiratory arrest prior to ventilation.

Relation to relapse and remission

In the present series respiratory complications were associated with clinical relapses in 17 patients and an acute deterioration in a progressive course in two patients. Guthrie *et al.* (1952) described four patients with acute respiratory failure which occurred during relapses: two of these patients were successfully intubated and ventilated but two patients died before support could be provided. All of the patients described by Guthrie *et al.* (1952) presented with dyspnoea, tachypnoea and tachycardia with reduced chest and diaphragm excursion, falling vital capacity and hypoxia. Neurologically, bulbar function was relatively normal but quadriparesis and high segmental sensory levels indicated the presence of spinal-cord lesions, involving pyramidal tracts bilaterally and possibly the anterior horn cells in the cervical and thoracic cord. In the present series the acute respiratory insufficiency was associated with high cervical cord lesions in 17 patients and bulbar lesions in 12 patients.

Respiratory muscle weakness

Respiratory muscle weakness had been increasingly recognized as a cause of respiratory insufficiency in a variety of neuromuscular and myopathic disorders (Ferguson *et al.*, 1982; Howard *et al.*, 1988, 1989). This often leads to nocturnal alveolar hyperventilation causing hypersomnolence, morning headache and sleep disturbance particularly if diaphragm weakness predominates (Newsom Davis *et al.*, 1976). Fourteen of our patients had clinical evidence of respiratory muscle weakness and six of these also had marked diaphragm weakness. Most patients had symptoms of progressive breathlessness, orthopnoea and sleep disturbance before developing acute respiratory failure.

In paraparetic patients with MS, expiratory muscle weakness increases as the upper extremities become involved; the presence of concomitant bulbar dysfunction increases the likelihood of aspiration and aspiration pneumonia (Smeltzer *et al.*, 1988). Weakness of the expiratory intercostal muscles was associated with a reduced ability to cough in three patients. In five other patients, coexisting bulbar disease exacerbated the risk of aspiration leading to lower respiratory tract infection in four of them. Weakness of abdominal muscles will also reduce the capacity to cough as does abdominal distension caused by ileus, constipation and bladder distension; all factors which disadvantage the paraplegic patient.

Diaphragmatic weakness in MS usually occurs as part of generalized respiratory muscle weakness, although it may be predominant. Six patients had significant diaphragm weakness: four had orthopnoea and two severe sleep disturbance. They all had paradoxical diaphragm movement on inspiration with a significant reduction of vital capacity in the supine position in each case. All the patients had clinical evidence of high cervical cord lesions. Details of localization were available in five cases, four from MRI and one from autopsy examination. In all cases there was severe involvement of the medulla and cervical cord to the C7 level. The characteristic feature of diaphragm weakness, namely a relatively preserved erect vital capacity with a marked decline on adopting the supine posture, implies relative preservation of the upper motor neuron (willed) drive to inspiratory muscles other than the diaphragm. The presence of such a pattern may thus indicate either bilateral lesions in phrenic upper motor neurons or selective involvement of the ventromedial cell column of the anterior horns of C3 – C5, the origin of the phrenic lower motor neurons. Severe reduction in vital capacity independent of posture implies extensive high demyelination of descending medullary and cervical (upper motor neuron) pathways or involvement of respiratory lower motor neurons in the cervical and thoracic cord. The available clinical, MRI and autopsy evidence in our cases suggests multiple level involvement.

Disordered regulation of breathing and bulbar dysfunction

The voluntary respiratory pathway operates during wakefulness and allows behavioural modulation of respiration. The system depends on willed activity from the cerebral cortex passing via the corticospinal tracts and descending in the dorsolateral columns of the spinal cord (Plum, 1970; Aminoff and Sears, 1971). Interruption of the voluntary pathways in man leads to an unusually stable ('autonomous') respiratory pattern during wakefulness as well as in sleep, and loss of the ability to take a deep breath, to breath hold, to cough voluntarily or to initiate any kind of volitional respiratory movement (Plum, 1970; Newsom Davis, 1974). However, tidal volume remains responsive to CO_2 and a reflex cough is preserved. Newsom Davis (1974) described a 38-yr-old man with autonomous breathing of this type resulting from a subacute pattern transverse myelitis in the region of the cervicomedullary junction. In the present series three patients had a similar though less severe syndrome in which voluntary control was compromised. All had brainstem and upper cervical cord lesions causing quadriplegia. The severity of the loss of voluntary control of respiratory muscles paralleled that of limb muscles, suggesting that this system is served by descending tracts that lie together in the dorsolateral columns. Our data do not allow us to exclude the possibility that abnormalities of voluntary control could be ascribed entirely to respiratory muscle weakness.

The automatic system controls ventilation to maintain acid-base status and oxygenation. Impaired automatic control of ventilation is suggested by severe hypoventilation or apnoea during sleep or a highly variable pattern of tidal volume or frequency (Baker *et al.*, 1950; Severinghaus and Mitchell, 1962; Mellins *et al.*, 1970; Rizvi *et al.*, 1974). One severely disabled patient in this series developed episodes of nocturnal hypoventilation and apnoea requiring intubation and ventilation. She had evidence of an anterior spinal-cord plaque suggesting involvement of the descending automatic system in the ventrolateral region of the cord, in close relationship to the spinothalamic tracts, possibly in the descending reticulospinal tracts (Nathan, 1963).

Lesions in the region of the dorsolateral pons may affect respiratory timing, in particular the termination of inspirations. Lesions in this region may induce apneustic breathing with sustained inspiratory cramps and a prolonged pause at full inspiration or alternating brief end-inspiratory and expiratory pauses (Plum and Alvord, 1964). Two patients had apneustic breathing in association with bulbar or spinal-cord relapses. Both patients suffered frequent episodes of abnormal breathing patterns although respiratory support was not required in either case. A further patient had a pattern of ratchet breathing in addition to apneustic breathing. In ratchet breathing there are irregular and jerky inspiratory breaths with short apnoeic pauses during mid-inspiration, this is thought to be a feature of localized lesions in the medulla (Plum and Posner, 1980).

Failure of the automatic system is often associated with acute bulbar lesions although this only occurred in one patient in the present series. The combination of an impaired swallowing mechanism, abnormalities of the respiratory pattern, reduced vital capacity and reduced or absent triggering of a cough reflex all increase the risk of aspiration pneumonia. Respiratory insufficiency was associated with bulbar weakness in nine patients and six of these had evidence of lower respiratory tract infections that was due to aspiration. The presence of hiccuping in two patients is a particularly important sign of medullary involvement in the region of the nucleus tractus solitarius intimately associated with respiratory control (Newsom Davis, 1970).

Clinical signs of lesion of the ninth and tenth cranial nerves include loss of posterior pharyngeal wall sensation, reduced palatal movement and pharyngeal reflex, poor cough and impaired speech and swallowing (Newsom Davis, 1985). However, clinical signs of bulbar dysfunction are not always a good guide to the development of aspiration (Linden and Siebens, 1983; Splaingard *et al.*, 1988). In three patients, lower respiratory tract infections, thought to be due to aspiration, occurred in the presence of an apparently adequate pharyngeal reflex and swallow.

One patient suffered paroxysmal flexion spasms precipitated by hyperventilation (Twomey and Espir, 1980). However, two patients had episodes of paroxysmal hyperventilation in association with an acute brainstem relapse. There were clear ictal features in one patient and the attacks were thought to be due to the tonic seizures which occur in brainstem MS (Matthews, 1958, 1975). It remains possible that the hyperventilation attacks were due to abnormalities of automatic control or were psychogenic in origin.

Respiratory support

As with other causes of neuromuscular weakness, respiratory insufficiency may develop insidiously with few or no symptoms before the onset of respiratory failure. If respiratory failure is associated with progressive bulbar weakness the airway must be protected by endotracheal intubation with a cuffed tube to prevent aspiration and allow respiratory support with intermittent positive pressure ventilation. If bulbar failure or respiratory insufficiency initially fail to resolve, a tracheostomy may be necessary; subsequent improvement may allow weaning from respiratory support and the use of unprotected airways (e.g. silver tube) until the tracheostomy can be closed. With respiratory insufficiency in the presence of normal bulbar function, ventilation may be assisted by negative or positive pressure support. Increasing use of nasal or mouthpiece IPPV (Carroll and Branthwaite, 1988) may be helpful in managing patients with MS who develop respiratory impairment. However, the effect of this technique on any tendency to aspirate will need to be clarified particularly in patients with bulbar dysfunction and impaired cough.

When embarking on mechanical support for breathing in MS our data suggest that weaning may often not be possible (six out of 12 patients ventilated). On the other hand, six patients were weaned and three of these within a fortnight. The chances of weaning might be construed as better if respiratory deterioration occurs in the context of a clear clinical relapse rather than if mechanical support has been used in a gradually declining situation. However, our data do not provide clear evidence on this point.

The present series of 19 patients with MS and major respiratory complications emphasizes the multifactorial nature of the respiratory impairment in this disease. A majority of patients (14) had significant respiratory muscle weakness (global or predominantly diaphragm) due either to involvement of descending respiratory pathways or respiratory motor neurons in the spinal cord. However, the effect of such weakness on lung function was compounded by several other factors, notably concomitant bulbar dysfunction (and resultant aspiration) and disorders of regulation of breathing. A smaller group presented primarily with evidence of bulbar failure or disorders of respiratory pattern, suggesting dominant pathology in the pons and medulla. In most patients more than one major factor seems to have been present.

Early recognition of patients at risk of acute respiratory complications is important because the provision of appropriate support during the period of respiratory insufficiency reduces the incidence of sudden death, may allow the resumption of spontaneous ventilation with remission of the relapse and may allow survival for prolonged periods with or without respiratory support. The series serves to emphasize that temporary ventilatory support, either nocturnal or continuous, may be necessary during acute bulbar and spinal-cord relapses of MS. However, with adequate airway protection and ventilation, some patients improve significantly with remission and not all require continued mechanical assistance once this is commenced.

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PRIMARY LATERAL SCLEROSIS

CLINICAL FEATURES, NEUROPATHOLOGY AND DIAGNOSTIC CRITERIA

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SUMMARY

Eight patients with a homogeneous syndrome of progressive symmetric spinobulbar spasticity were studied. Clinical features were limited to those associated with dysfunction of the descending motor tracts and included spastic quadriparesis, pseudobulbar affect, spastic dysarthria, hyper-reflexia and bilateral Babinski signs. Lower motor neuron findings were absent and higher cognitive function preserved. Median age of onset was 50.5 yrs and median disease duration was 19 yrs. Neuropathologic features (including morphometric analysis) in the single autopsied case confirmed the selective involvement of the motor cortex. There was complete absence of Betz cells from layer 5 of the precentral cortex and the remaining pyramidal cells were significantly smaller than those seen in normal controls. Magnetic resonance imaging (MRI) revealed atrophy of the precentral gyrus and positron emission tomography (PET) scans showed diminished glucose [^{18}F]fluorodeoxyglucose uptake in the pericentral cortex. Magnetic motor cortex stimulation revealed markedly prolonged central motor conduction times. The literature is reviewed and diagnostic criteria for primary lateral sclerosis based on clinical, laboratory and imaging features are proposed.

INTRODUCTION

Since the first descriptions in the 19th century (Charcot, 1865; Erb, 1875), the existence of primary lateral sclerosis (PLS) as a distinct entity has been challenged. Much of the early literature appears to include cases of Strumpell's disease, amyotrophic lateral sclerosis (ALS), unrecognized spondylitic myelopathy or spinal multiple sclerosis (MS). In recent years, however, a handful of reports using modern diagnostic techniques and pathologic verification have supported the existence of PLS as a distinct, albeit rare, clinical disorder (Fisher, 1977; Beal and Richardson, 1981; Younger *et al.*, 1988).

Since the duration of the disease is long, it is unsatisfactory to await pathological evidence prior to making the diagnosis. Prognostic considerations make earlier diagnosis important. It has been suggested recently that, if proper investigations are utilized, a clinical diagnosis is permissible (Russo, 1982; Sotaniemi and Myllylä, 1985; Gastaut *et al.*, 1988). We here report the clinical features of eight cases of PLS, the neuropathological findings in one case, and suggest diagnostic criteria for this entity to replace those put forward prior to the availability of modern diagnostic techniques (Stark and Moersch, 1945).

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MATERIALS AND METHODS

Ascertainment

Eight patients with a syndrome of progressive spinobulbar spasticity were identified and followed regularly through neurologic clinics at the University of Western Ontario, London, Ontario. All but two patients were initially thought to have chronic myelopathy due to multiple sclerosis (MS). These patients were identified over a period of 10 yrs among a population of some 2000 patients carrying a diagnosis of MS (Weinshenker *et al.*, 1989) and 500 patients of ALS (Hudson *et al.*, 1986). Recognized causes of a progressive spastic motor syndrome were systematically sought.

Investigations

All patients underwent complete blood count, thyroid, liver and renal function tests, serum glucose, calcium, magnesium, phosphate, folate and vitamin B_{12} determinations, as well as serum protein electrophoresis, sedimentation rate, rheumatoid factor, antinuclear antibody titres and serologic tests for syphilis. Serology and gene amplification studies for human T-cell lymphotrophic virus, type 1 (HTLV-1) were performed in six patients (Power *et al.*, 1989). Human immunodeficiency virus (HIV) serology was not performed because no patient had risk factors for HIV infection. All patients underwent HLA typing. Cerebrospinal fluid (CSF) parameters were checked in all patients, and in seven out of eight patients the fluid was examined for oligoclonal bands (Ebers, 1984). In the remaining patient, a colloidal gold curve had been carried out.

Electromyography (EMG) was carried out on representative proximal, intermediate and distal limb muscles in at least one arm and leg. If fibrillation or positive sharp waves were found, a careful search was carried out for similar activity in other muscles as a means of establishing the limits of the denervation. Fibrillation was graded in a manner similar to that reported by Miller *et al.* (1988). Nerve conduction studies were carried out in all patients.

Imaging studies

Compressive spinal lesions were ruled out by appropriate procedures [myelograms and/or magnetic resonance imaging (MRI) of the cervical spine] in all cases. All patients underwent cranial MRI with a General Electric Signa 1.5 Tesla scanner. On paramedian sagittal images, the central sulcus was identified as the sulcus anterior to the marginal sulcus, a constant landmark which bends upwards in continuity with the cingulate sulcus (Steinmetz *et al.*, 1989). The precentral and postcentral gyri were identified medially by thus locating the central sulcus, and inferiorly by locating the anterior ascending ramus of the lateral sulcus (Ebeling *et al.*, 1989). Scans were compared with age-matched ALS, demented and normal controls (n = 7, 10, 14, respectively). The widths of the precentral gyrus and the central sulcus (each measured on paramedian sagittal images at the level of the maximum width of the central sulcus) were measured and mean values calculated for all patients and controls. The means were compared using analysis of variants and Duncan's multiple range test.

Positron emission tomography (PET scan) was performed in three patients using i.v. [^{18}F]fluorodeoxyglucose 1 h before scanning. Maps of cortical [^{18}F]fluorodeoxyglucose uptake were constructed using the method described by Garnett *et al.* (1990).

Central motor conduction was assessed in seven of our eight patients by stimulating the motor cortex with a magnetic coil (Cadwell MES-10). The coil was centred just ahead of the vertex and the intensity and direction of the current flow set to produce maximum motor evoked potentials from the hypothenar and tibialis anterior muscles while the patient attempted moderate voluntary contration of these muscles. Ten motor evoked potentials were superimposed. Central motor conduction times were calculated by subtracting the latencies from electrical stimulation (DEVICES D18O stimulator) at C6 for hypothenar muscles and L1 for tibialis anterior from the latencies to the same muscles in response to magneto-electrical stimulation of the motor cortex.

Autopsy and morphometric studies

Post-mortem examination was carried out on patient 5 after 3 wks of fixation of the brain and spinal cord in phosphate buffered picrate-formaldehyde (Stefanini *et al.*, 1967). Blocks were taken from many areas including frontal, temporal and occipital cortex, hippocampus, diencephalon, thalamus, striatum, midbrain, pons and medulla. The precentral gyri were identified and blocks taken from the left and right precentral gyri corresponding to the homuncular regions of hand, face and leg. All levels of the spinal cord were examined. Histological stains included haematoxylin and eosin (H&E), Nissl, Bielchowsky, periodic acid Schiff, phosphotungstic acid and haematoxylin (PTAH), Congo red, and myelin stains. Immunohistochemical staining with antisera against glial fibrillary acidic protein (GFAP) (Dako) was also employed.

For morphometric comparisons with the case of PLS, specimens of spinal cord (level L4), medulla (level of the hypoglossal nucleus) and the left and right precentral gyri (foot, hand and tongue areas) were collected from seven patients who died with the sporadic form of amyotrophic lateral sclerosis. The duration of the disease ranged from 4 mths to 5 yrs from diagnosis. Specimens from the same parts of the central nervous system were also collected from six controls, who died from diseases that did not involve the nervous system. The controls and ALS patients were selected from a larger collection on the basis of the ages at death being fairly close to the age (71 yrs) of the case of PLS. The specimens were taken from brains and spinal cords that had been fixed in neutral, phosphate-buffered 4% formaldehyde or in phosphate-buffered picrate-formaldehyde (Stefanini *et al.*, 1967) for 4 wks, and subsequently stored in neutral 4% formaldehyde for up to 3 yrs. Paraffin section 20 μm thick were prepared and stained by Einarson's chrome alum gallocyanine method (Kiernan, 1990) to show Nissl substance and nuclei. The slides were given code numbers in the hope of reducing subjective bias, but as in an earlier study of this kind (Kiernan and Hudson, 1991) it was not possible to conceal the conspicuous pathological changes in the spinal cords of the ALS cases.

Using a camera lucida, sections through the ventral horns and the hypoglossal nuclei were drawn on paper at a linear magnification of ×25. Motor neurons were marked and counted. Individual motor neurons with their nucleoli in the plane of section were traced at ×400. Pyramidal neurons (all the triangular or pear-shaped cells with nucleoli) in layer 5 in sections of cerebral cortex were also drawn at ×400. It was assumed that the nucleolus was in the centre of each neuron. A Micro-plan II Digital Image Analyzer (Donsanto Corporation, Natick, MA) was used to measure the areas of the drawn profiles. Repeated measurements of individual cells indicate a reproducibility of ±2% for motor neurons and ±7% for small cortical neurons in the hands of the investigator (J.A.K.) who made the measurements. The abundance of motor neurons was recorded as the number of the cells per 20 μm section of anterior horn or hypoglossal nucleus at each level. No attempt was made to determine numbers of neurons in the cortical specimens.

For the ALS and control groups, the mean, standard deviation (SD) and standard error (SEM) were determined for the number of motor neurons per 20 μm section of ventral horn or hypoglossal nucleus (the left and right sides were pooled). For each type and location of motor and pyramidal neuron, the mean area with its SD and SEM was calculated for each individual subject. Group means of the mean areas of motor neurons and pyramidal cells were calculated for comparison of the cases of ALS with the controls. For each measured parameter, a one-sample Student's t test was used to compare the mean for each of the ALS or control groups with the single value obtained from the case of PLS. The computations were made using the Number Cruncher Statistical System, Version 5.03, 1990 (J. L. Hintze Software, Kaysville, UT). For all statistical comparisons, a value of $P < 0.05$ was considered significant to be due to chance (Zar, 1984).

CASE REPORTS

Case 1

A 70-yr-old woman with no significant past or family history was well until 1986 (age 66 yrs) when she developed dysarthria. Shortly thereafter she began to note cramping of both legs. By 1987 she had a severe spastic dysarthria and was noticing stiffness of both legs as well as bilateral hand weakness. By 1988 she was essentially anarthric but could communicate through writing. She began to notice bouts of unprovoked laughing and crying, but noticed no change in her mental capacity. She had no pain, visual disturbance, L'Hermitte's phenomenon, sensory complaints nor bowel or bladder dysfunction. Dysphagia developed in 1989. By 1990 she required assistance with walking.

Her general examination in 1990 was unremarkable apart from the neurological findings. Neurologically, she was cognitively intact. Her visual fields were full but there was saccadic breakdown of smooth pursuit. She had a spastic facies with bilateral palmomental reflexes. Jaw jerk was abnormally brisk, as was the gag reflex. Movements of the tongue were slow. Motor examination revealed profound spasticity of all limbs. There was slight weakness more evident in the extensors of the upper extremity and flexors of the lower extremities. Wasting and fasciculations were absent from the tongue, trunk and limbs. Reflexes were brisk and the Babinski sign was present bilaterally. Sensory and cerebellar tests were normal. Gait was markedly spastic.

Electromyography in 1988 showed no evidence of denervation but in 1990, occasional fibrillation potentials were found in pronator quadratus, thenar and the interosseous muscles only. The CSF was negative for oligoclonal bands. Magnetic resonance imaging scan of the cervical spine showed no compressive lesions. Cranial MRI showed cerebral atrophy, most striking in the precentral gyrus. Magnetic cortical stimulation showed no response in spite of adequate motor unit recruitment.

Case 2

A 68-yr-old woman with no significant past history or family history was well until 1984 (age 62 yrs) when she noticed 'clumsiness' of both legs. Her arms were initially not affected. She had no complaints of pain, paresthesiae, L'Hermitte's phenomenon, visual change or sphincter dysfunction. Her gait gradually worsened and she began to experience frequent falls. Four years from onset, she had begun to note progressive speech difficulties, and required assistance in walking. In early 1989 she began to notice difficulty with fine finger movements as well as bouts of inappropriate laughing and crying. In March of 1988 (at age 66 yrs) she suffered a myocardial infarction but made a good recovery.

When evaluated in 1990, her general physical examination was normal apart from the neurological findings. Gait was markedly spastic. She displayed a severe spastic dysarthria. Mental status, visual fields and acuity were normal. There was minor breakdown of smooth pursuit eye movements. Gag reflex was brisk. Movements of the tongue were slow but atrophy, fasciculations or weakness were not apparent. Pout and palmomental reflexes were present. Motor examination revealed no wasting or fasciculations of the extremities. There was marked spasticity in the arms and legs, but power was preserved. Reflexes and jaw jerk were brisk and the Babinski sign was present bilaterally. Sensory and cerebellar examinations were normal.

Electromyography and nerve conduction studies in 1987 and again in 1989 were completely normal, with no evidence of denervation. The CSF was negative for oligoclonal bands. Somatosensory evoked potentials (SSEPs) were normal. Magnetic resonance imaging of the cervical spine showed no evidence of spondylosis or foramen magnum abnormality. Cranial MRI showed cerebral atrophy most pronounced in the region of the precentral gyrus (Fig. 5). Magnetic stimulation of the motor cortex evoked no motor responses in spite of apparently normal motor unit recruitment.

Case 3

In 1981 a 50-yr-old Italian man with no significant past history or family history noted weakness and clumsiness of his left leg. Within a month, he had a marked gait disturbance with weakness and 'stiffness' of both legs, as well as clumsiness of both arms. Over the following months, his difficulties progressed and he developed dysarthria, dysphagia, dysphonia and emotional lability. He had no sensory complaints, L'Hermitte's phenomenon nor visual symptoms. By 1983, he required assistance in walking and by 1986 he was confined to a wheelchair. His dysarthria had worsened and he had developed urgency incontinence. At the time of his last evaluation (in 1990) there had been further progression. He was virtually immobilized by severe spasticity. His speech was rendered nearly incomprehensible by profound spastic dysarthria; however, he was able to communicate via eye movements and monosyllabic responses.

His general examination in 1990 was normal apart from the neurological findings. He was cognitively intact. His visual fields were full. There was saccadic breakdown of smooth pursuit. He had a spastic facies with bilateral palmomental and pout reflexes as well as emotional incontinence. Gag reflex was brisk and there was spasticity of the tongue (without wasting or fasciculation). Motor examination showed severe spastic quadriparesis without wasting or fasciculations. Deep tendon reflexes were extremely brisk in all limbs, with sustained clonus in the lower extremities. Sensory and cerebellar function was normal.

Complete urodynamic studies supported an obstructive uropathy. Electromyograms in 1982, 1986 and 1990 showed no evidence of denervation. The CSF was negative for oligoclonal bands. Visual, auditory and somatosensory evoked responses were completely normal as were myelograms, post-myelogram CT and cranial CT. Magnetic resonance imaging showed mild cerebral atrophy most pronounced in the region of the precentral gyrus. The PET scan was indistinguishable from normal. Magnetic motor cortical stimulation evoked a very small amplitude motor evoked potential from the hypothenar muscles. The central motor conduction times to the hypothenar and tibialis anterior muscles were greatly prolonged (two and three times the upper limit of normal, respectively).

Case 4

A 59-yr-old woman with no significant past history noted difficulty running in 1981 (at age 50 yrs). She had no sensory complaints, L'Hermitte's phenomenon,, visual disturbance nor bowel or bladder dysfunction. There was no family history of neurologic disease. Over the following 2 yrs, she gradually developed dysphagia, dysphonia and dysarthria. By 1983, she required assistance walking and by 1987 was confined to a wheelchair. She had episodes of inappropriate laughing and crying and had become essentially anarthric. Communication was achieved through eye blinking. When evaluated in 1990, she had recently developed urinary incontinence.

General examination was unremarkable apart from the neurological findings. Neurological examination in 1990 showed her to be cognitively intact. Visual fields were full. Smooth pursuit eye movements were broken down into saccades. She had a spastic facies with pout and palmomental reflexes. She was anarthric but vocalization was possible. Gag reflex was brisk. Movements of the tongue were slow but there was no wasting and no fasciculations. Motor examination revealed profound spasticity of all limbs with sustained clonus of all reflexes and a Babinski sign bilaterally. Wasting and fasciculations were absent. Sensory and cerebellar tests were normal.

Electromyography nerve conduction studies, as well as visual and SSEPs were all normal. The CSF was negative for oligoclonal bands. Computerized tomography and myelograms of the head were normal. Cranial MRI showed cerebral atrophy most prominent in the frontoparietal region. Positron emission tomography scans showed a decreased glucose uptake in the pericentral cortex (Garnett *et al.*, 1990). Magnetic motor cortex stimulation showed no response.

Case 5

A 71-yr-old Italian man with no significant past or family history first noted stiffness and weakness of his legs in 1975 (at age 57 yrs). Within a year he was also noticing clumsiness of his hands and by 1977 he had developed spastic dysarthria and emotional lability. His symptoms gradually worsened and within 4 yrs of onset he was confined to a wheelchair. By 1983 his dysarthria was severe and by 1987 he was anarthric. He began to note urgency and hesitancy, both of which symptoms improved following a transurethral resection of his prostate. When last evaluated in 1990, he was again having difficulties initiating his urinary stream. However, he was not incontinent. He had no sensory complaints, no visual disturbance and no L'Hermitte's phenomenon.

On examination in 1990, his general examination was normal apart from the neurological findings. He was cognitively intact. Visual fields were normal but there was saccadic breakdown of smooth pursuit. Testing of cranial nerve functions demonstrated a brisk gag reflex and spasticity of the tongue (without wasting or fasciculations). Pout and palmomental reflexes were present. Motor examination revealed marked spasticity of all limbs with no focal wasting or fasciculations. Extremity movement was confined to hand grips and forearm flexion bilaterally. Reflexes were brisk bilaterally with a bilateral Babinski sign. Sensory and cerebellar exams were normal.

An electromyogram in 1987 was normal but in 1990 showed occasional fibrillation potentials in the first dorsal interosseous, tibialis anterior and extensor digitorum brevis muscles. Pelvic EMG and urodynamic testing were not performed. The CSF was negative for oligoclonal bands. Myelograms were normal. Cranial computerized tomography (CT) was unremarkable. Magnetic resonance imaging detail was limited by motion artefact. Magnetic motor cortical stimulation evoked no detectable motor responses.

In 1990 the patient developed abdominal pain and was admitted to hospital where he subsequently died of a massive gastrointestinal haemorrhage. A full autopsy was performed.

Case 6

A 52-yr-old Italian woman with no significant past history or family history first noted isolated left-hand weakness in 1973 at the age of 35 yrs. Her hand weakness progressed and by January 1975 was bilateral. By 1977 the weakness in her upper extremities had progressed and she had developed weakness and spasticity of both legs. She had no visual symptoms, no disturbance of bowel or bladder function, no sensory symptoms and no L'Hermitte's phenomenon. Dysphagia developed in 1979. By 1981, 8 yrs into her disease, her hands were practically useless and her gait was markedly spastic. Speech began to be affected with a spastic dysarthria. Intellect was preserved. By 1983, she was confined to a wheelchair and was anarthric. Inappropriate laughing and crying were noted.

On last examination in 1987, her general examination was unremarkable apart from the neurological findings. She remained cognitively intact with full visual fields. She had a spastic facies and brisk gag reflex. Her tongue showed neither wasting nor fisciculations. She was virtually immobile with a severe spastic quadriparesis. Reflexes were brisk and there was a Babinski sign bilaterally. There was no wasting and no fasciculations of the limbs. Sensory and cerebellar examinations were normal.

Electromyography in 1973, 1975, 1983 and 1987 showed no evidence of denervation. The CSF was negative for oligoclonal bands. Visual and somatosensory evoked responses were normal. Pneumoencephalogram and cerebral angiograms in 1973 were normal. Myelograms in 1973 and 1977 were normal. Cranial CT was unremarkable. Cranial MRI in 1987 showed cerebral atrophy most striking in the region of the precentral gyrus (Fig. 6). PET scan showed a 50% reduction in glucose consumption in the pericentral cortex bilaterally (Garnett *et al.*, 1990).

Case 7

A 67-yr-old man with no significant past or family history was well until 1967 when, at the age of 44 yrs, he first noted 'catching' of his left leg. Over the following year his symptoms progressed to involve his right leg, and by 1972 he had involvement of all four limbs. By the mid-1970s he had developed a spastic dysarthria. His symptoms progressed slowly but steadily and when last evaluated in 1990 he was still able to walk slowly with canes. His hands were clumsy and he was incapable of fine finger movements. He described bouts of unprovoked laughing and crying but noted no change in his mental capacities. He had no pain, sensory symptoms, L'Hermitte's phenomenon, or bowel or bladder disturbance.

His general physical examination was unremarkable apart from the neurological findings. He was cognitively intact but had a severe spastic dysarthria. Visual fields were full and eye movements were normal. He had a somewhat spastic facies with brisk jaw jerk and bilateral palmomental reflexes. Gag reflex was brisk. There was spastic weakness of the tongue without wasting or fasciculation. There was spasticity of all limbs but strength was well preserved. Wasting and fasciculations were absent in the limbs. Reflexes were brisk with sustained clonus at the knees and ankles as well as spread and cross of reflexes. There was a Babinski sign bilaterally. Gait was markedly spastic. Sensory and cerebellar tests were normal.

Electromyograms in 1972, 1987 and 1990 showed no evidence of denervation. Visual and somatosensory evoked responses were normal. The CSF was negative for oligoclonal bands. A myelogram in 1972 showed a C6 spondylitic bar which was removed surgically without altering his symptoms. Cranial MRI showed focal atrophy in the precentral region. Magnetic motor cortical stimulation showed greatly prolonged central motor conduction times, over two and three times the control upper limit for the hypothenar and tibialis anterior muscles, respectively.

Case 8

A 73-yr-old man with no significant past or family history first came to medical attention in 1956 at the age of 39 yrs with complaints of stiffness and weakness of the legs and arms. In retrospect, he may have had lower extremity symptoms as early as age 21 yrs when he noted mild weakness during intensive military training. His symptoms progressed and by 1965 he was confined to a wheelchair with a spastic quadriparesis. He began to note dysarthria soon thereafter which had progressed to anarthria by 1986. He had no sensory complaints, no L'Hermitte's phenomenon and no visual symptoms. He developed urinary incontinence in approximately 1980. In 1989 he suffered an uncomplicated myocardial infarct and made a full recovery.

He was last evaluated in 1990. His general examination was unremarkable apart from the neurological findings. Although anarthric, he was able to write slowly in response to questions. He was cognitively intact. Visual fields were full but there was saccadic breakdown of smooth pursuit. He demonstrated emotional lability. Cranial nerves were normal apart from facial spasticity, brisk gag reflex and spasticity of the tongue (without wasting or fasciculations). Palmomental and pout reflexes were present. Motor examination revealed a spastic quadriparesis of all limbs. Wasting and fasciculations were absent. Reflexes were brisk and there was sustained clonus at the knees and ankles. A Babinski sign was present bilaterally. Sensory and cerebellar examinations were normal.

Electromyograms (most recently in 1990) showed no evidence of denervation. Visual evoked responses were normal. The CSF analysis (including colloidal gold curve) was normal. Pneumoencephalograms and myelograms were normal. Cranial MRI showed mild cerebral atrophy, most prominent in the precentral region. Magnetic cortical stimulation revealed greatly prolonged central motor conduction times (over twice the control upper limit) for both hypothenar and tibialis anterior muscles.

RESULTS

Clinical features

Clinical data are summarized in Table 1. These eight unrelated Caucasian patients consisted of four males and four females with median age of onset of symptoms being 50.5 yrs (mean 50.4 yrs, range 35–66 yrs). Three of our patients (Cases 3, 5, 6) were Italian by birth. Onset of insidiously progressive spastic paraparesis occurred in all but two patients (onset with dysarthria and spastic arm paresis). All cases progressed to symmetric spinobulbar spastic paresis. None of our patients had a family history of neurologic disease. No common occupational or environmental exposure was identified.

Findings were limited to those associated with dysfunction of descending motor tracts: spastic quadriparesis, hyperactive deep tendon reflexes, a bilateral Babinski sign, spastic dysarthria and emotional lability (namely, pseudobulbar affect) were seen in all patients. Muscular atrophy and fasciculations were uniformly absent. Occasional fibrillation and positive sharp waves as indicators of relatively minor degrees of denervation were found in two patients and in both cases were restricted to a few muscles and appeared late in the course of the disease, in the setting of immobility secondary to severe spasticity (Brown and Snow, 1990). Intellect was preserved in all cases. Saccadic breakdown of smooth pursuit eye movements was noted in five patients. Although four patients remained free of bladder involvement at last evaluation, the remaining four developed urinary symptoms as a late feature (5, 8, 9, 24 yrs after initial symptoms).

All our patients derived some benefit from antispasticity medications (Dantrolene, Lioresal), although none had a sustained (i.e. >1–2 yrs) response. Amitriptyline was helpful for control of pseudobulbar features. In all cases, the progression has been slow but inexorable, with a median disease duration of 19 yrs (mean 14.5 yrs, range 4–34 yrs) at the time of last evaluation.

Neuropathologic findings (Case 5)

The general autopsy revealed a massive gastrointestinal bleed from a chronic pyloric ulcer but was otherwise unremarkable. The brain weighed 1066 g. Cortical atrophy

TABLE 1. SUMMARY OF CLINICAL DATA

Case	*Sex*	*Age of onset (yrs)*	*Duration (yrs)*	*Site of onset*	*Urinary symptoms*	*Intellect preserved*	*Speech dysarthria*	*Emotional lability*	*Saccadic breakdown*	*Time to wheelchair (yrs)*
1 (M.G.)	F	66	4	Speech	–	+	+	+	+	>4 (SA)
2 (D.B.)	F	62	6	Legs	–	+	+	+	+	>6 (SA)
3 (J.P.)	M	50	9	Left leg	+	+	+	+	+	5
4 (C.B.)	F	50	9	Legs	+	+	+	+	+	6
5 (G.P.)	M	57	15	Legs	+	+	+	+	+	4
6 (G.S.)	F	35	17	Left arm	–	+	+	+	+	10
7 (L.B.)	M	44	23	Legs	–	+	+	+	–	>23 (SA)
8 (H.S.)	M	39	34	Legs	+	+	+	+	+	9

F = female; M = Male; + = present; – = absent; ? = not recorded; SA = still ambulant.

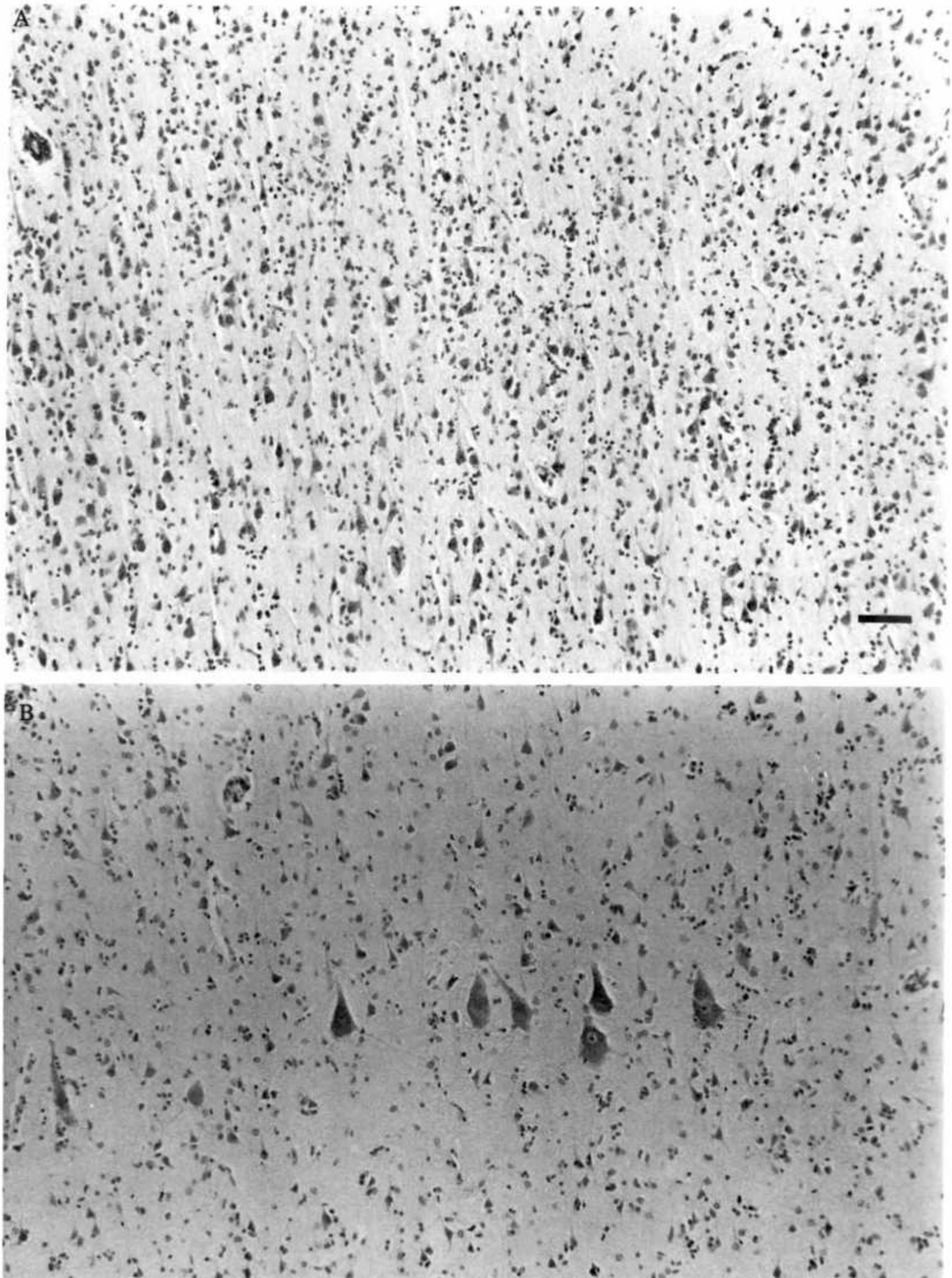

FIG. 1A,B

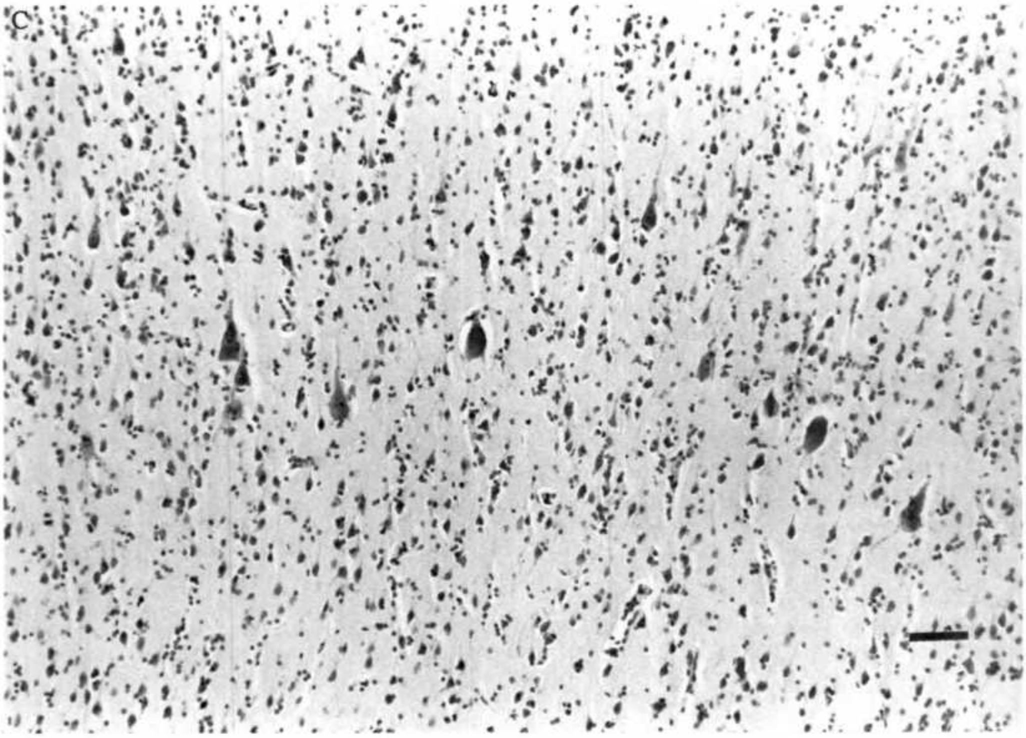

FIG. 1. Precentral gyrus of Case 5 (A) compared with normal (B) and ALS (C), showing complete absence of Betz cells in PLS (Nissl stain). Bar = 40 μm.

was most prominent in the region of the central sulcus. The precentral gyri 1 cm from the inter-hemisphere fissure measured 0.5 cm and 0.4 cm across (left and right, respectively), compared with 1.2 cm and 1.0 cm for adjacent regions of the postcentral gyri. At 3.5 cm from the inter-hemisphere fissure, the precentral gyri measured 0.7 cm and 0.8 cm across compared with 1.0 cm for both postcentral gyri. The remainder of the hemispheres appeared normal. The base of the pons appeared slightly flattened and the pyramids of the medulla appeared smaller than normal. The spinal cord, anterior and posterior roots all appeared normal.

Microscopically, the most striking feature was the complete absence of Betz cells (pyramidal cells longer than 50 μm) from layer 5 and a decreased number of pyramidal neurons in layers 3 and 5 of the cortex of the precentral gyri (Fig. 1).The increased cellularity in the PLS case appeared to result from decreased neuropil and gliosis. The remaining cortical neurons showed no cytoplasmic swelling, no Lewy bodies, no Pick bodies, and no neurofibrillary tangles. Glial fibrillary acidic protein stains showed laminar gliosis in layers 3 and 5 of the cortex of the precentral gyri. These changes had a tendency to merge gradually in the depths of the sulci adjacent to the precentral gyrus with normal cytoarchitecture of adjacent gyri (including postcentral gyrus) rather than having a distinct margin. Other regions of the neocortex showed no abnormality.

As shown in Table 2, morphometric analysis revealed that in the case of PLS there

TABLE 2. NUMBERS AND SIZES OF MOTOR NEURONS AND CORTICAL PYRAMIDAL CELLS OF THE PRECENTRAL GYRUS, IN NORMAL CONTROLS, ALS CASES AND ONE CASE OF PLS

				Significance of difference between	
	Normal control	*ALS*	*PLS*	*PLS and normal control*	*PLS and ALS*
No. of cases	6	7	1		
Age at death (yrs)	74+4.2	69+2.5	71	n.s.	n.s.
Motor neurons					
L4 motor neurons per ventral horn per 20 μm section	49+4.4	23+5.0	39	n.s.	n.s.
L4 motor neurons means area of cells sectioned through nucleolus (μm^2)	1484+195	1008+199	1672	n.s.	$P < 0.02$
Hypoglossal neurons per nucleus per 20 μm section	39+2.1	18+5.9	29	n.s.	n.s.
Hypoglossal neurons mean area of cells sectioned through nucleolus (μm^2)	1077+35	990+111	1068	n.s.	n.s.
Cortical neurons					
Precentral pyramidal cells: foot area. Mean area, cells with nucleoli (μM^2)	579+56	395+37	326	$P < 0.02$	n.s.
Precentral pyramidal cells: hand area. Mean area, cells with nucleoli (μm^2)	452+59	383+16	278	$P < 0.04$	$P < 0.01$
Precentral pyramidal cells: tongue area. Mean area, cells with nucleoli (μm^2)	400+57	337+13	223	$P < 0.03$	$P < 0.001$

Values are Mean±EM. n.s. = not significant ($P < 0.05$).

was a considerable reduction in the mean area of pyramidal neuronal cell bodies in the precentral gyri. Pyramidal cells in layer 5 of the precentral gyrus are normally largest on the medial surface of the hemisphere, in the area concerned with the foot, and smallest near to the lateral sulcus, in the area concerned with the face and mouth. This trend was also seen in ALS and in the single case of PLS. However, the cortical neurons of all sizes were significantly smaller in PLS than in the normal or ALS controls (*see* Table 2), except in the foot area, for which the ALS cases did not differ from the PLS case. The absence of very large pyramidal cells (Betz cells) from the primary motor cortex does not greatly influence the mean neuronal size because the giant cells constitute only a small fraction of the population (Lassek, 1941). In PLS there is a general shrinkage of neurons in the precentral gyrus, and the change is more marked than in ALS.

Sections from the internal capsule showed mild myelin pallor in the posterior third. Sections from the basal ganglia showed some calcification of the walls of small vessels in the globus pallidus but were otherwise unremarkable. The sections from the hippocampi showed no neuronal loss, neuritic plaques or neurofibrillary tangles. Sections from the midbrain showed a normal number of pigmented cells in the substantia nigra with no gliosis other than moderately severe gliosis of the periaqueductal grey commonly seen in aged individuals. The pontine sections were remarkable only for mild pallor of the descending fibres in the basis pontis. Neurons in the facial and trigeminal motor nucleus appeared to be of normal number and size. This was also true of the lateral vestibular nucleus where the lateral vestibulospinal tract originates. None of these nuclear groups demonstrated gliosis. Sections from the medulla showed pallor of both pyramids but were otherwise unremarkable; specifically, the neurons of the motor nuclei were normal in number and appearance (Fig. 2). Morphometric analysis of the hypoglossal nuclei confirmed this finding (Table 2). Sections from the cerebellum were normal.

Sections from the spinal cord showed degeneration in the dorsolateral quadrant of the white matter at all levels as well as some pallor of the fasciculus gracilis (Fig. 3) and sulcomarginal fasciculus as well as in the ventral part of the ventral funiculus in a location usually assigned to the vestibulospinal tract. In spinal segments S2 and S3 the sacral autonomic nucleus and nucleus of Onufrowicz were of normal appearance. There was rarefaction of the neuropil of the anterior horns. Phosphotungstic acid and haematoxylin and GFAP staining showed gliosis in the anterior horns, most marked in the cervical region. The anterior horn cells were normal in number at all levels (Fig. 4). Occasional individual cells in the lumbar region showed some swelling and dissolution of Nissl substance in the pattern of central chromatolysis. However, atrophic cells, Lewy body-like inclusions and Bunina bodies were not seen. Lumbar and hypoglossal motor neurons were analysed morphometrically and the results are shown in Table 2. It can be seen in the case of PLS that the numbers of motor neurons did not differ significantly from the mean numbers determined in the normal controls. Although the number of motor neurons in the PLS case was greater than the number of ALS controls, the difference did not reach significance (Table 2). This was probably due to the amount of variation among the six cases of ALS: two did not have paralysed tongues, and two others had only mild involvement of the lower limbs. The motor neurons in the case of PLS were larger than those of the ALS cases in segment L4.

The nucleus of Onufrowicz is a distinctive group of neurons in the most ventral part of the ventral horn in segment S2. It is believed to supply the muscles of the pelvic floor. Morphometric analysis of the nucleus of Onufrowicz yielded no significant difference between cases of ALS, PLS and normal controls, with 5 neurons per side per 20 μm section in the PLS patient compared with a mean of 6.0 in ALS (SEM = 0.4) and 6.7 in normal controls (SEM = 2.2). Similarly, the mean area of neurons in the nucleus of Onufrowicz was 531 μm^2 compared with a mean of 551 in ALS controls (SEM = 73) and 547 in normal controls (SEM = 142).

Sections from peripheral nerves (common peroneal and ulnar) were normal.

Cranial MRI

Seven out of eight patients had satisfactory cranial MRI with no evidence of neoplasm, infarction or foramen magnum lesions in any case. In no cases were high signal areas such as those seen in MS present. The most striking feature was the presence of atrophy which differed from the degree and distribution of atrophy found in control subjects (age matched ALS, normal and demented subjects). Gyral detail was obscured by motion artefact in Case 5 but in six cases (1, 2, 3, 6, 7, 8), the atrophy was most marked in the precentral gyrus (Figs 5, 6). In all cases, atrophy was most prominent in the

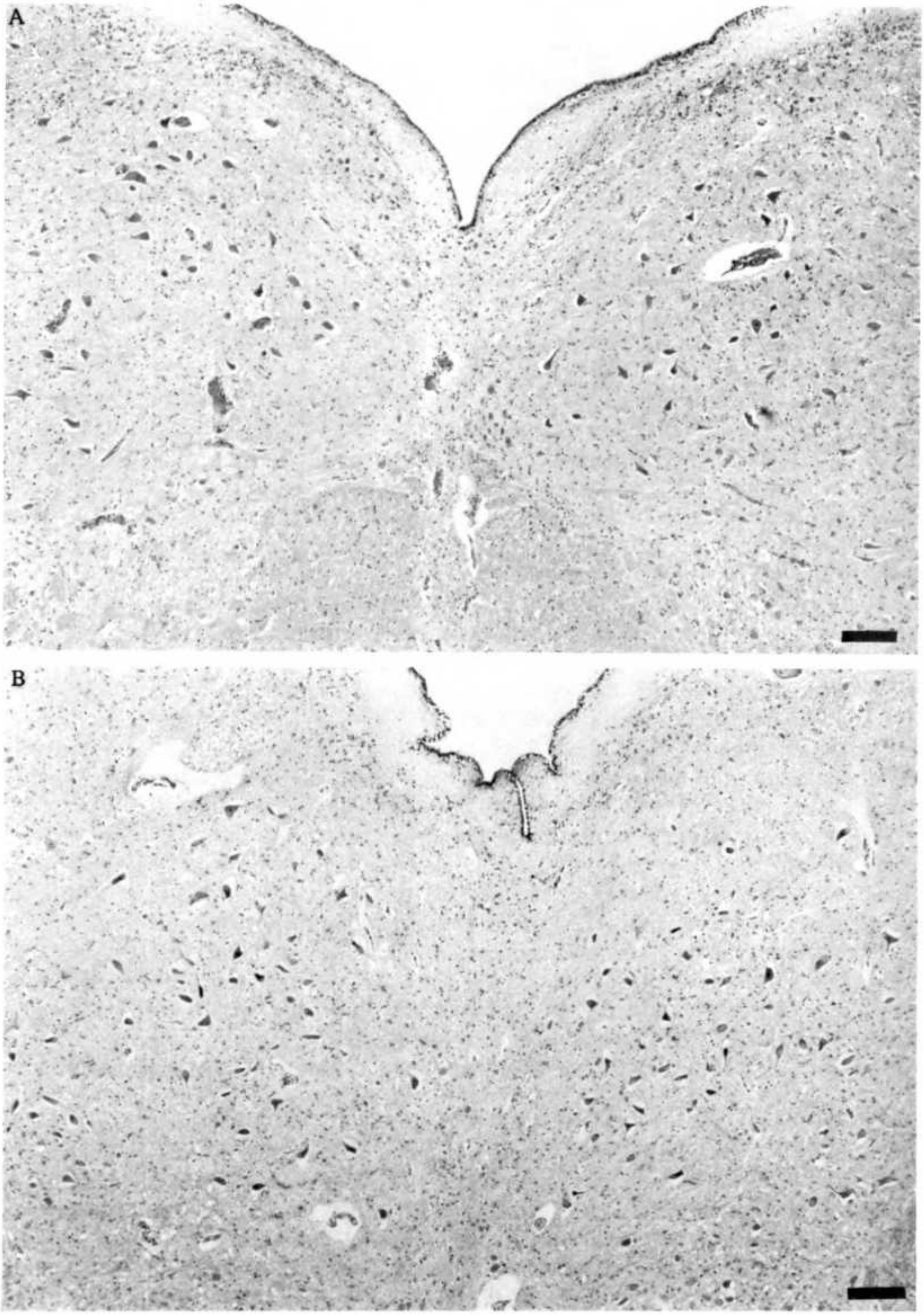

FIG. 2. Hypoglossal nuclei of Case 5 (A) compared with normal (B) (H&E). Bar = 150 μm.

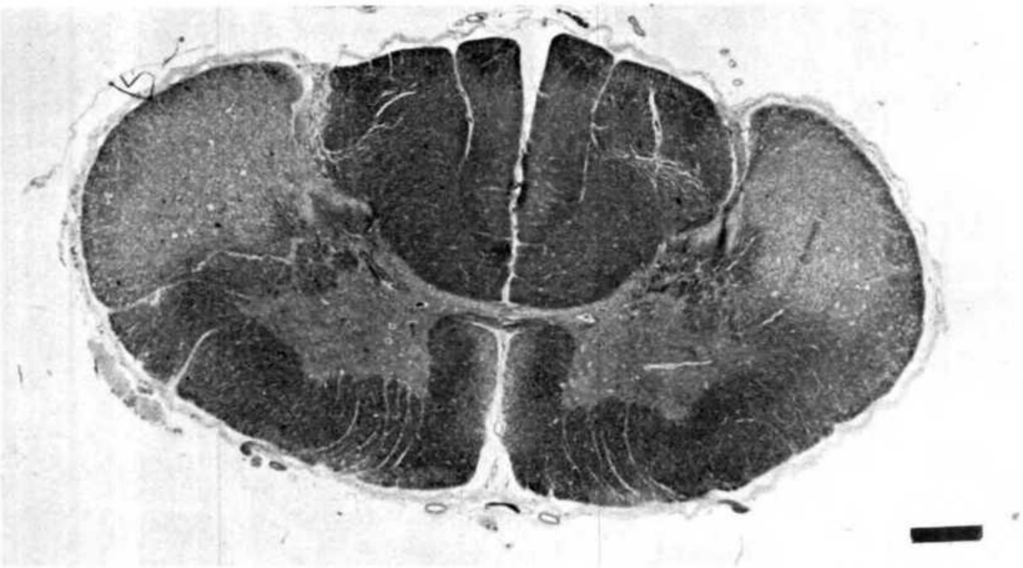

FIG. 3. Whole mount of spinal cord of Case 5 showing pallor of corticospinal tracts (myelin stain). Bar = 1 mm.

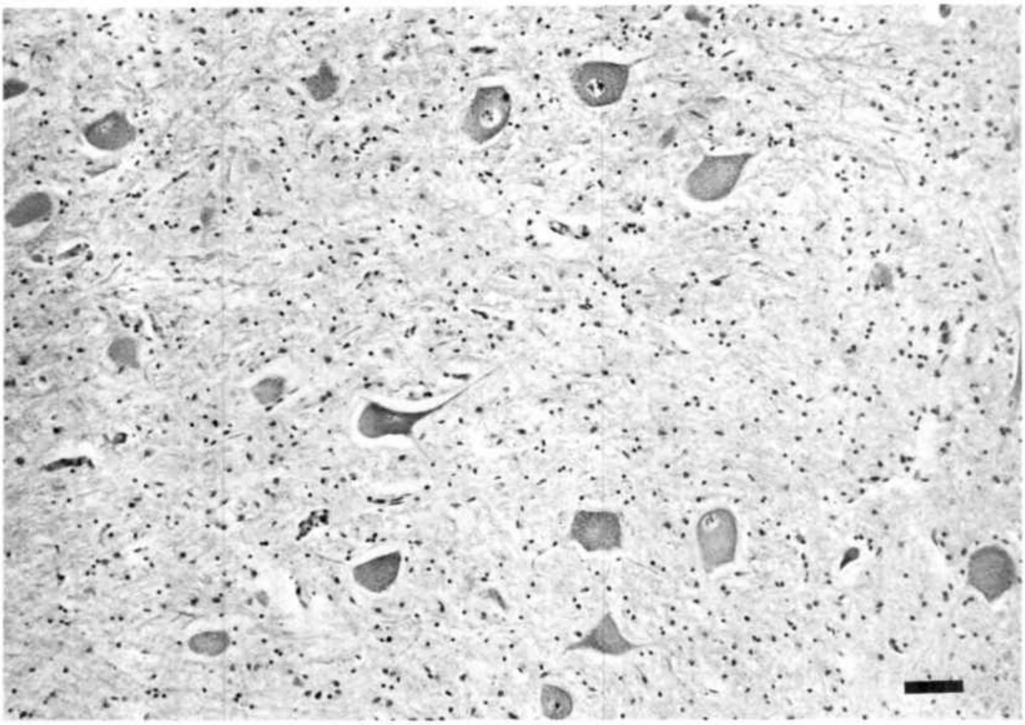

FIG. 4. Lumbar anterior horn cells of Case 5 (H&E). Bar = 40 μm.

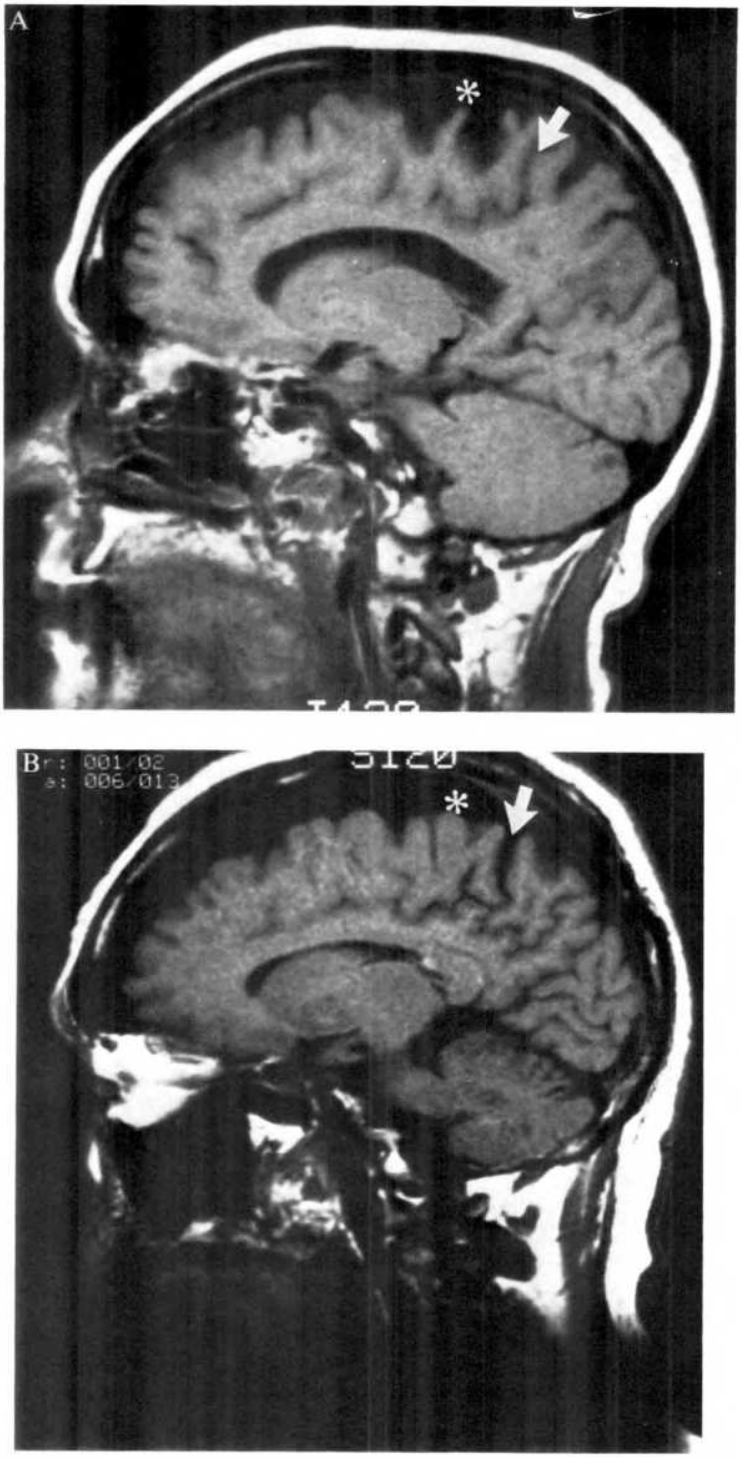

FIG. 5A,B

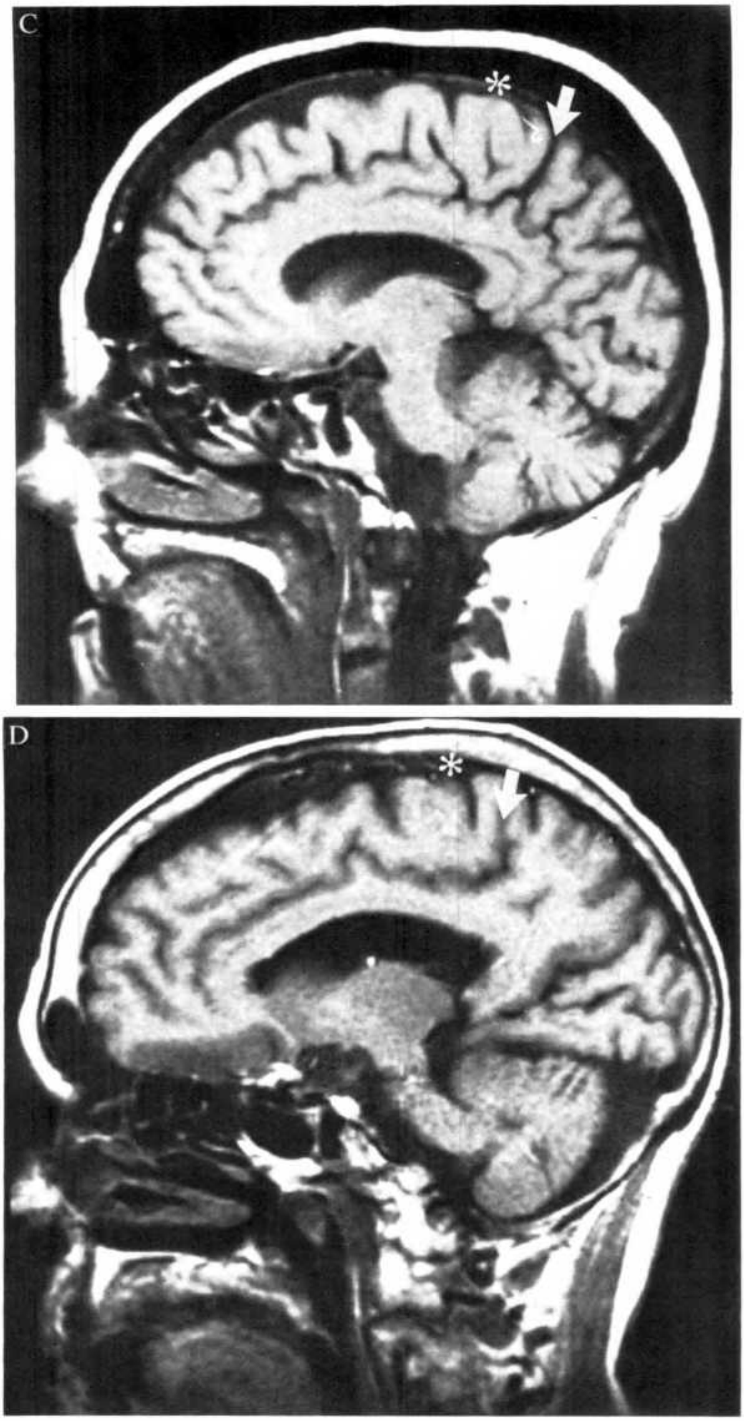

FIG. 5. Sagittal T_2-weighted images (10 mm to right of midline) of PLS patient (Case 2, A) compared with age-matched normal (C), demented (B) and ALS (D) controls, showing atrophy of precentral gyrus in PLS patient (TR 500, TE 20). Arrow = marginal sulcus; * = precentral gyrus.

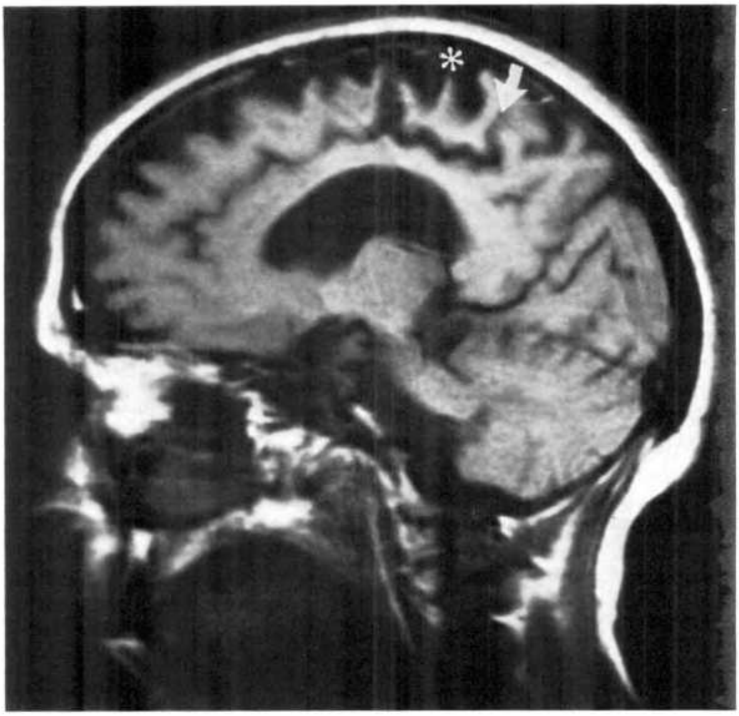

FIG. 6. Sagittal, T_2-weighted image (7.5 mm to left of midline) of Case 6, showing atrophy of precentral gyrus (TR 500, TE 20). Arrow = marginal sulcus; * = precentral gyrus.

frontoparietal regions. The width of the central sulcus in PLS patients was found to be significantly larger than normal, ALS and demented controls. Similarly, the width of the precentral gyrus was found to be significantly smaller in PLS patients than in controls (Table 3). No difference was found between the mean widths of the postcentral gyrus in PLS patients and normal controls. Slightly increased signal intensity on T_2 weighted images was seen in the posterior limb of the internal capsule in both PLS and those ALS patients with predominantly upper motor neuron features.

PET scans

Three patients underwent PET scanning and results have been previously reported (Garnett *et al.*, 1990). In Cases 4 and 6, [^{18}F]fluorodeoxyglucose uptake in the pericentral cortex was diminished. Case 3 (less severely affected than the others at the time of scanning) was indistinguishable from the normal and age-matched ALS controls.

Magnetic motor cortex stimulation

Seven of our patients underwent motor cortex stimulation. All had normal peripheral motor conduction times (i.e. spinal roots to motor points). In four of the cases (1, 2, 4, 5) we were unable to elicit motor evoked responses to cortical stimulation despite the fact that two (Cases 1, 2) of the patients were able to strongly abduct the thumb. The remaining patients were unable to voluntarily abduct the thumb. In the Cases (3,

TABLE 3. MAGNETIC RESONANCE IMAGING DATA

	Mean widths ± standard deviation	
	Precentral gyrus	*Central sulcus*
PLS (n = 7)	1.6±0.7*	3.1±0.7*
Normal (n = 14)	3.5±1.0	1.2±0.4
ALS (n = 7)	4.0±1.2	1.7±0.9
Demented (n = 10)	4.5±1.2	1.7±0.7

* Significant difference using Duncan's multiple range test ($P = 0.05$).

7 and 8) who showed motor evoked responses, the central conduction times were markedly prolonged (more than two times the latency of any control). Case 7 was the least affected of those undergoing magnetic motor cortex stimulation (i.e. still ambulant).

DISCUSSION

Review of the literature

Although the term PLS was coined by Erb in 1875 (Erb, 1875), the syndrome of slowly progressive spastic quadriparesis with sclerosis of the lateral columns had been described much earlier by Charcot who referred to two earlier cases described by Turck in 1856 (Charcot, 1865). However, it was Erb who proposed that PLS represented a nosologic entity distinct from the recently delineated ALS (Charcot and Joffroy, 1869). Erb later reviewed 10 pathologically verified cases (Erb, 1902). Of these, four (Cases 2, 6, 7, 8) would now be classified as the hereditary spastic paraparesis of Strumpell (Strümpell, 1886). As well, Case 9, who presented atypically with pain and 'signs of chronic alcoholism', was contaminated by the associated pathological finding of 'congenital hydromyelus in the lumbar and dorsal cord'. All the remaining five patients, however, had clinical and pathological findings confined to the corticospinal tracts. The clinical syndrome described by Erb consisted of a spastic paraparesis (of gradual onset and steady progression) with increased reflexes and Babinski signs without sensory signs or bladder disturbance. Erb emphasized that the disease was not directly fatal, but rather tended to be of long duration, the patient finally dying 'in a marasmic condition'.

Two further pathologically verified cases were published by Spiller (1905) and a third by Buzzard and Barnes (1906) in which pseudobulbar emotional incontinence was a prominent feature. However, there were no further pathologically supported cases forthcoming for many years. Swank and Putnam included 21 patients with probable PLS in their clinical analysis of ALS patients (Swank and Putnam, 1943). Stark and Moersch followed 43 cases which they felt were clinically consistent with the diagnosis of PLS (Stark and Moersch, 1945). Clinical details were given for only eight of the cases. Wechsler and Brody described 15 cases of spastic paraparesis which they termed PLS in spite of the presence in some of them of sensory signs (Wechsler and Brody, 1946). Given that none of the cases in either of these series were pathologically verified, the unavailability of EMG to rule out subclinical lower motor neuron involvement and myelography to rule out spondylitic myelopathy makes the cases suspect.

This suspicion was shared by a number of authors who challenged the existence of PLS as a distinct entity, citing the lack of modern pathological confirmation and suggesting that the syndrome represented a forme fruste of ALS in which lower motor neuron findings would always eventually appear (Wilson, 1940; MacKay, 1963). A number of pathological studies of ALS cast further doubt on the existence of PLS. Lawyer and Netsky, looking at 53 cases of ALS, found no cases which met the anatomic criteria for PLS (Lawyer and Netsky, 1953). In a clinicopathological study of 45 cases of ALS, Brownell identified three cases (Cases 39, 40, 41) which clinically appeared to be PLS (Brownell *et al.*, 1970), but in which necropsy revealed involvement of the lower motor neurons. The duration of symptoms before death in all three was short, being 22 mths, 16 mths and 30 mths, respectively. Case 40 had associated dementia and anomia. None of the three cases was said to have been examined with EMG to detect subclinical denervation. Similarly, Castaigne's group looked at the pathology of two cases of 'clinical PLS' in their study of 61 ALS patients (Castaigne *et al.*, 1972) and found typical features of ALS. However, both cases should clearly have been identified clinically as predominantly pyramidal ALS, as pyramidal signs, although prominent, were not isolated.

In a number of studies authors have tried to address the problem of chronic spastic paraparesis and its relation, if any, to PLS (Marshall, 1955; Hübbe and Mouritzen Dam, 1973; Ungar-Sargon *et al.*, 1980; Sotaniemi and Myllylä, 1985). The diagnosis in 25 of Marshall's 52 patients remained obscure after 10 yrs of follow-up. However, on pathological exam, when the aetiology of the paraparesis in each of these cases was identified, there was none with PLS. In other series, 15 out of 255 patients (Hübbe and Mouritzen Dam, 1973) and 44 out of 672 patients (Ungar-Sargon *et al.*, 1980) with chronic spastic paraparesis, the diagnosis remained obscure after a long follow-up. Pathological examination of these cases was not available, however, and we would be reticent to label patients without bulbar involvement or with a nonprogressive illness as having PLS.

The first pathologically verified case of PLS of the modern era was a man who developed progressive spinobulbar spastic paresis at the age of 67 yrs (Fisher, 1977). All clinical features were felt to be attributable to lesions in the corticospinal tract, and EMGs and myelography were used to rule out denervation and spondylosis, respectively. The patient suffered a right-sided pontine infarct during the course of his disease but pathological examination showed, in addition to the pontine lesion, pallor and atrophy of the corticospinal tracts from the medulla to the cord (with sparing of the anterior horn cells and cranial motor nuclei). There was also pallor of the fasciculus gracilis in the cervical region. The giant pyramidal cells of the motor cortex (Betz cells) were noted to be 'probably reduced in number'. Fisher reported five other cases with a clinical picture of spinobulbar spasticity as well as two other cases of less certain diagnosis: one of spastic quadriparesis without bulbar involvement in whom the disease process was static, and one of an isolated spastic paraparesis with cervical spondylosis on myelogram.

The best pathologically documented case of PLS in the literature to date is that of a 67-yr-old woman who presented with a spastic dysarthria (Beal and Richardson, 1981; *see* Table 4). Clinically, this case was comparable with those reported in our series. She died 3 yrs after onset and pathologic examination showed striking focal atrophy of the precentral gyrus bilaterally. Microscopic examination revealed complete loss of Betz cells from layer 5 of the cortex of the precentral gyrus, with decreased numbers of pyramidal neurons in layers three and five and associated gliosis. The corticospinal

TABLE 4. PATHOLOGICALLY VERIFIED CASES OF PRIMARY LATERAL SCLEROSIS

(Modern Literature)

Reference	*Sex*	*Age of onset*	*Duration (yrs)*	*Site of onset*	*Bladder involvement*	*Intellect preserved*	*Speech affected*	*Emotional lability*	*Time to wheelchair (yrs)*	*Pathology*	*Comment*
Fisher (1977)	M	67	5	Legs	?	+	+	+	?	Old infarct basis pontis. Motor cortex Betz probably decreased. Degeneration CST throughout.	Complicated by pontine infarct
Beal and Richardson (1981)	F	61	3.5	Speech	–	+	+	+	?	Striking atrophy of PCG and pyramid. Loss of Betz cells, PCG with marked gliosis. Degenerative CST throughout.	Few eosinophilic inclusions in anterior horn cells
Younger *et al*. (1988)	M	65	6	Left leg	–	?	+	?	?	Demyelination of internal capsule, middle cerebral peduncle and crossed CST of cord. Betz cells normal.	Metastatic adenocarcinoma of lung discovered at autopsy
Younger *et al*. (1988)	M	55	11	Legs	?	–	+	?	?	Demyelination of CST medulla to cord, normal at higher levels (incl. PCG). Several cystic areas right caudate, thalamus and frontal gyrus.	C6–7 ridge on myelogram infarcts
Pringle *et al*. (1990)	M	57	15	Leg	+	+	+	+	4	Loss of Betz cells PCG with marked gliosis. Degeneration CSTs internal capsule to cord. Gliosis anterior horn with preserved number of motor neurons.	

CST = corticospinal tract; PCG = precentral gyrus.

tract throughout the length of the neuraxis showed loss of myelinated fibres. Other systems were unaffected as were the motor neurons of the cranial nerves and anterior horns.

Younger *et al.* (1988) reported a clinically heterogeneous collection of patients which included cases with lung cancer, denervation on EMG, cervical spondylosis, a fourth ventricle ependymoma and HIV seropositivity. None of their three autopsied cases showed the abnormalities of Betz cells or gliosis of the precentral gyrus seen in previous autopsy studies and confirmed in the present report. However, one of their cases (Case 2) had EMG evidence of loss of motor units, denervation and re-innervation, as well as short disease duration (13 mths), features which would lead us to exclude it as a case of PLS. Of their other two cases, one was complicated by the finding of metastatic adenocarcinoma at autopsy and the other had multiple infarcts (right caudate, frontal, thalamus). They cite one of their living cases as an example of 'transition from PLS to ALS'. The case, however, clearly had EMG features which would exclude the diagnosis of PLS and indeed the patient developed weakness and fasciculations within 3 yrs of onset. In our personal experience, we have not seen the late clinical development of lower motor neuron involvement (cases of multisystem atrophy excluded).

Russo (1982), in his electrophysiologic studies of four patients with PLS, noted frequent bladder involvement, a finding in contrast to previous reports. He suggested that detrusor hyper-reflexia and small volume bladder are consistent with pyramidal tract dysfunction (Johnson *et al.*, 1989).

Gastaut reported five cases of spinobulbar spasticity in whom other diagnoses had been excluded on clinical and laboratory grounds (Gastaut *et al.*, 1988). He suggested, as had Russo, that a clinical diagnosis of PLS is permissible.

Present cases

Our cases appear to conform to a relatively homogenous clinical syndrome of isolated progressive corticobulbospinal dysfunction. The onset is insidious, usually in the fifth or sixth decade, the distribution among the sexes appears to be equal, and the findings are exclusively those which are traditionally associated with corticospinal tract dysfunction. We recognize this is an oversimplification of the complex pathways involved in motor performance and tone but use this term in its common and imprecise clinical context. In the cortex, the neuropathological features of Case 5 are similar to the previously reported cases with striking loss of Betz cells from layer 5 of the precentral cortex and decreased numbers of pyramidal cells from layers 3 and 5 of the precentral cortex with accompanying laminar gliosis. Our study has also made it apparent that there is shrinkage of pyramidal neurons of all sizes in PLS. A similar change, though of generally smaller magnitude, has been found in ALS. In the latter disease, the diminution in cortical neuronal size is not correlated with the distribution of lower motor neuron damage, and it has been suggested that the cortical changes occur independently of those in the brainstem and spinal cord (Kiernan and Hudson, 1991). The reductions of mean cortical pyramidal neuronal area due to disease (changes of 15–30% in ALS and 38–44% in PLS) are too large to be attributable to inaccurate measurement or to variations in the thicknesses of paraffin sections cut at a microtome setting of 20 μm (Aherne and Dunnill, 1982).

The spinal cord findings in Case 5 are identical to those of the previously described cases; that is, degeneration in the dorsolateral half of the lateral funiculus and in the medial and ventral parts of the ventral funiculus. These regions of the white matter contain all the corticospinal fibres and also many fibres that descend from the central group of pontine and medullary reticular nuclei (the classical reticulospinal tracts), the vestibular nuclei (vestibulospinal tract and descending component of the medial longitudinal fasciculus) and the raphe nuclei of the brain-stem. All these tracts influence movement and muscle tone. Quantitative and morphometric data have not previously been reported in PLS nor has the gliosis of the anterior horn. Given the preservation of anterior horn cell numbers, we feel that this gliosis may reflect loss of descending afferent fibres. Changes in anterior horn cells in ALS usually consist of atrophy and the presence of Bunina bodies (Brownell *et al.*, 1970; Kiernan and Hudson, 1991), neither of which were present in our cases of PLS. Swollen neurons have also been reported (Munoz *et al.*, 1988). The finding of central chromatolysis in some of the lumbar anterior horn cells may reflect trans-synaptic degeneration.

The finding of bladder dysfunction in half of our patients is worthy of further comment. Although the patients in the present series who developed bladder symptoms had this as a relatively late feature, it was not possible to be certain that these were an intrinsic feature of PLS. We cannot disprove the claim that bladder dysfunction may be present in PLS; however, we would emphasize that the development of bladder disturbance early in the disease was not seen, and if present should raise suspicion of another diagnosis or independent cause. Furthermore, we did not identify any pathological correlate of bladder dysfunction of our autopsied case in which both the sacral autonomic and Onufrowicz nuclei were intact. This finding is similar to that in ALS in which condition the bladder is not affected (Mannen *et al.*, 1977, 1982; Schrøder and Reske-Nielsen, 1984). Although detrusor hyper-reflexia and small volume bladder are known to be often associated with corticospinal tract dysfunction (Johnson *et al.*, 1989), localization within this tract is imprecise. We cannot rule out the possibility that bladder symptoms in our patients may have been due to the disease process itself.

Until our initial communication (Pringle *et al.*, 1990), previous authors had reported normal MRIs in PLS (Gastaut *et al.*, 1988; Younger *et al.*, 1988). In a recent report by Martí-Fàbregas and Pujol (1990), the authors pointed out increased signal in the posterior limb of the internal capsule on T_2-weighted images in a patient with PLS. We also saw this in the MRIs of our patients, but it was not helpful in distinguishing them from those ALS controls in whom pyramidal features were prominent. All of our patients, however, had atrophy, particularly of the frontoparietal area, and in the majority this was more striking within the precentral gyrus. This was not seen in randomly selected, age-matched control cases. Therefore, we suggest that MRI demonstrates a previously unrecognized imaging correlate of the precentral gyrus atrophy described on gross pathology (Beal and Richardson, 1981).

Our conclusion that PLS is a restricted motor system degeneration sparing lower motor neurons is supported by the fact that all patients remained cognitively intact, as judged by the neuropathological findings and by normal SSEPs in those patients who underwent the test. The failure to delineate a distinct anterior margin of atrophy on the MRI of Case 4 and the neuropathology of Case 5 may be due to a tendency of cortical area 4 to melt gradually into area 6 (Freund *et al.*, 1985). Nevertheless it must be realized that clear-cut MRI change in the precentral gyrus may not be present at the initial diagnostic evaluation when it would be of most value.

To our knowledge, magnetic cortical stimulation studies have not previously been reported in PLS. As these motor evoked responses are normally transmitted by rapidly conducting pyramidal tract fibres, the absence of a motor evoked response especially in the two patients able to strongly abduct the thumb, suggests direct involvement of the descending cortical neuronal fibres (Amassian *et al.*, 1987; Rossini, 1988). Our findings support disease of the corticospinal tracts but are nonspecific for PLS, similar results having recently been reported in patients with ALS (Eisen *et al.*, 1990). However, the prolongation of central conduction seen in our cases was much longer than that reported in ALS. Details of these data are the subject of another paper by our group (Brown *et al.*, 1992).

Neither the present study nor the literature to date allow valid conclusions regarding the exact incidence of PLS; however, the syndrome would appear to be far more rare than ALS. That this syndrome exists is undeniable although its exact relationship to the other motor neuron diseases is unclear. Certainly, there are features which seem to separate PLS from the other motor neuron diseases. Mean disease duration (at the time of last evaluation) in our study was 14.9 yrs, whereas the average disease duration in ALS in our experience is 2.5 yrs from the date of diagnosis (Hudson *et al.*, 1986). This striking difference in prognosis is perhaps the most compelling reason to attempt to separate PLS from ALS. Our study also suggested differences in laboratory findings; specifically, MRI and PET scans were different in the PLS patients compared with ALS controls. However, it is possible that these differences are only reflections of disease duration, since the Betz cell loss and shrinkage and/or loss of other neurons and neuropil that would seem to be the underlying pathological substrate for the imaging abnormalities is seen to some degree in ALS (Brownell *et al.*, 1970). The pathological changes in anterior horn cells also serve to separate the two entities.

It is not clear that PLS represents a homogeneous entity, although there is some clinical and pathological support for this notion. Whether PLS simply represents one end of the spectrum of motor neuron disease (with progressive muscular atrophy at the other end) or whether it is indeed a completely separate disease is a question which cannot be conclusively answered until the aetiology and pathogenesis of the motor neuron diseases are elucidated. However, we favour the latter interpretation. At present, the cause of PLS remains obscure, although a number of hypotheses has been suggested over the years. Parallels between PLS and lathyrism have been drawn since the time of Erb (Erb, 1902; Minchin, 1940; Spencer *et al.*, 1984). The identification of a glutamate-related neurotoxic amino acid, beta-(*N*)-oxalyl-L-alanine, as the chemical likely responsible for lathyrism (Spencer *et al.*, 1986) is especially interesting in light of the recent finding of elevated levels of a metabolite of *N*-acetylaspartyl-glutamate in the CSF of two patients with PLS (Rothstein *et al.*, 1990). The possibility of a genetically determined susceptibility to an environmental or toxic agent could also be postulated, but evidence is lacking. No common occupational exposure was found in this series.

The role of genetic factors in the development of PLS is unclear. Although a genetic cause might be readily dismissed in the absence of identified familial occurrence, the number of cases reported are as yet too few. It is not impossible that some rare conditions are accounted for by the interaction of two independent complementing recessive loci which would give a very low recurrence risk in siblings and which could be made even lower by late age of onset considerations. That these cases might represent sporadic cases of Strumpell's disease (hereditary spastic paraplegia) has been suggested (Younger *et al.*, 1988), but the eventually severe bulbar involvement is distinctly unusual in Strumpell's disease and pathological findings differ between the two entities (Sutherland, 1975; Baraitser, 1982).

Definitive treatment is unlikely to become available until the aetiology is determined. In the meantime, however, symptomatic relief is worth pursuing. As mentioned, most of our patients had brief responses to conventional doses of antispasticity medications (Lioresal and Dantrolene) but sustained responses were not seen. We have been impressed by the utility of Amitriptyline for relief of pseudobulbar emotional incontinence, a particularly distressing symptom to these cognitively-intact patients (Schiffer *et al.*, 1985).

Proposal of new diagnostic criteria

In light of our findings, we feel that re-examination of the diagnostic criteria for PLS (Stark and Moersch, 1945) is warranted. Given the protracted course and the severe degree of disability, the economic consequences to an individual may be devastating. Clearly, an early and accurate diagnosis is of value and has prognostic implications. In Table 5, we propose new diagnostic criteria which utilize modern diagnostic techniques.

In contrast to the criteria of Stark and Moersch, we would not exclude dysarthria and bladder involvement, although as discussed above, the latter appears to be a relatively late feature if it occurs. In addition, we feel that their arbitrarily chosen disease duration of 5 yrs is too restrictive. In fact, the duration of 5 yrs before diagnosis would exclude the previously best-documented pathologically verified case of PLS (Beal and Richardson,

TABLE 5. PROPOSED DIAGNOSTIC CRITERIA

Clinical

1. Insidious onset of spastic paresis, usually beginning in lower extremities but occasionally bulbar or in an upper extremity.
2. Adult onset, usually fifth decade or later.
3. Absence of family history.
4. Gradually progressive course (i.e. not step-like).
5. Duration ≥3 years.
6. Clinical findings limited to those usually associated with corticospinal dysfunction.
7. Symmetrical distribution, ultimately developing severe spastic spinobulbar paresis.

Laboratory (help in exclusion of other diagnoses)

1. Normal serum chemistry including normal vitamin B_{12} levels.
2. Negative serologic tests for syphilis (in endemic areas, negative Lyme and HTLV-1 serology).
3. Normal CSF parameters, including absence of oligoclonal bands.
4. Absent denervation potentials on EMG or at most, *occasional* fibrillation and increased insertional activity in a few muscles (late and minor).
5. Absence of compressive lesions of cervical spine or foramen magnum (spinal MRI scanning).
6. Absence of high signal lesions on MRI similar to those seen in MS.

Additionally suggestive of PLS

1. Preserved bladder function.
2. Absent or very prolonged latency on cortical motor evoked responses in the presence of normal peripheral stimulus-evoked maximum compound muscle action potentials.
3. Focal atrophy of precentral gyrus on MRI.
4. Decreased glucose consumption in pericentral region on PET scan.

1981). We feel that the diagnosis of PLS is permissible after 3 yrs, if other criteria are met as outlined in Table 2. Certainly, the other diagnostic considerations, such as ALS, should have become obvious within this period of time (Munsat, 1989), especially if careful examination for wasting and fasciculation is augmented by serial EMG studies to document the lack of significant subclinical lower motor neuron involvement. Other conditions such as the spastic paraparesis of Strümpell are ordinarily easily excluded, because of positive family history, although exclusion can be more confidently made after examining family members (even if asymptomatic). Careful attention to the course of the disease will eliminate many cases of MS and appropriate investigations (oligoclonal banding and MRI) should identify all but very few (Kempster *et al.*, 1987; Paty *et al.*, 1988). Radiologic investigations must be carried out to rule out spinal cord compression, especially when bulbar symptoms are absent.

We have included negative Lyme serology as a criterion in light of a recent communication from Sweden (Fredrikson and Link, 1988) describing a reversible pyramidal syndrome with high levels of antibodies against *Borrelia burgdorferi* in the cerebral spinal fluid.

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COGNITIVE FUNCTION AND REGIONAL CEREBRAL BLOOD FLOW IN PARKINSON'S DISEASE

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SUMMARY

In order to investigate relationships between cognition and regional brain function, we studied 20 non-demented patients with idiopathic Parkinson's disease (PD), 21 mildly demented patients with Alzheimer's disease (AD) and 24 control subjects using cognitive testing and single photon emission computerized tomographic (SPECT) measurements of relative regional cerebral blood flow (rCBF). Neuropsychological tests were grouped into clusters reflecting frontal lobe executive abilities, perseveration, memory and visuospatial ability, with a summary score summarizing performance in all four of these spheres. SPECT imaging utilized the tracer [^{123}I]*N*-isopropyl-*p*-iodoamphetamine with a relative measure of regional tracer uptake normalized to occipital radiotracer uptake (rCBF ratios). Patients with PD performed more poorly than controls in all cognitive domains, and were intermediate to AD patients and controls in tests of memory and overall cognitive functioning. Those PD patients who performed most poorly on neuropsychological testing showed lowest rCBF ratios in left and right temporal lobes. Using a stepwise multiple regression procedure, we examined patterns of correlations between cognitive clusters and predictor variables, including rCBF ratios, in the PD patients. We found that while patient age was a strong determinant of performance on the memory cluster and the summary score, dorsolateral frontal lobe perfusion and scores on a depression inventory accounted for a greater proportion of the variance of the frontal lobe and perseveration clusters than did age. These results imply that different neural mechanisms are responsible for the different aspects of cognitive decline seen in PD patients, with overall cognitive function closely related to age and temporal perfusion, while frontal lobe abilities are more linked to frontal perfusion and the presence of depression.

INTRODUCTION

Disturbed cognitive function is now a well-recognized feature of idiopathic Parkinson's disease (PD). These disturbances range from severe dementia to more circumscribed deficits in specific areas of cognitive function. Dementia occurs commonly, with estimates of its prevalence ranging from 10% to 35% (Brown and Marsden, 1984; Mayeux, 1984). In addition, many studies have noted the frequent occurrence of selective abnormalities of visuospatial function (Boller *et al.*, 1984), memory (Mohr *et al.*, 1990), and frontal lobe function (Lees and Smith, 1983; Taylor *et al.*, 1986*a*). Depression is also a frequent concomitant of PD (Taylor *et al.*, 1986*b*), and may independently affect cognitive function (Starkstein *et al.*, 1989). The wide spectrum of cognitive dysfunction in PD makes unitary descriptions of the pathophysiology of these cognitive deficits problematic.

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A common explanation for the development of the dementia syndrome in PD patients is that it may be caused by coexistent Alzheimer's disease (AD). Post-mortem studies of PD patients report the frequent occurrence of neuritic plaques and neurofibrillary tangles which are associated with the presence of dementia (Boller *et al.*, 1980). In addition, PD patients may show degeneration of the basal forebrain cholinergic system (Whitehouse *et al.*, 1983) which is also evidenced by AD patients. However, while the presence of AD may represent an important cause of dementia in PD patients it cannot be the sole explanation of severe cognitive decline in PD patients since parkinsonian patients with cognitive deficits seen during life have demonstrated a variety of different findings at autopsy (Chiu *et al.*, 1986; Torack and Morris, 1988) and do not invariably show Alzheimer-type neuropathology.

The lack of a consistent neuropathological explanation for the cognitive disturbances seen in PD patients had focused attention on the variability of neurobehavioural syndromes and their possibly different substrates. Of particular recent interest has been the predominance of abnormalities of frontal lobe function in PD patients. Early studies by Bowen (1976) demonstrated that PD patients were deficient on a variety of measures which were also failed by patients with focal lesions of frontal cortex. More recent studies of non-demented PD patients (Lees and Smith, 1983; Taylor *et al.*, 1986*a*) reported additional evidence of impairment on tests of 'executive' functions which are usually disrupted by frontal lesions. These abnormalities may also be present in demented parkinsonian patients (Pillon *et al.*, 1986). It is suggested that these frontal disturbances arise from either alterations in outflow of the caudate nuclei to frontal cortex, or from diminished dopamine in the frontal lobes consequent to degeneration of the frontal projections of the ventral tegmental area. This neurochemical or functional explanation for cognitive disturbance does not posit a structural or neuropathological lesion other than nigral degeneration. The evidence for this explanation is based largely upon the nature of the neuropsychological impairments in PD and upon the effects of levodopa replacement therapy on cognitive function, and has not been entirely consistent (Rafal *et al.*, 1984; Gotham *et al.*, 1988).

The development and application of functional brain imaging techniques such as positron emission tomography (PET) and single photon emission computerized tomography (SPECT) permits the study of cerebral physiology with a relatively high degree of spatial resolution. Studies of patients with AD have revealed marked disturbances of regional cerebral blood flow (rCBF) (Frackowiak *et al.*, 1981) and glucose metabolism (Friedland *et al.*, 1983; Duara *et al.*, 1986) which predominate in temporal and parietal cortex. Although many investigators have found increased blood flow and glucose metabolism in the basal ganglia of PD patients (Rougemont *et al.*, 1984; Wolfson *et al.*, 1985), findings in cerebral cortex have been more variable. Some reports have noted diminished global cerebral blood flow using the inhaled ^{133}Xe technique (Lavy *et al.*, 1979), while others have noted diminished frontal lobe flood flow using this technique as well as PET with [^{15}O]water (Bès *et al.*, 1983; Perlmutter and Raichle, 1985; Wolfson *et al.*, 1985). While these studies did not explore the relationship between these altered perfusion patterns and cognitive function, other PET and SPECT studies indicate that demented PD patients may show diminished temporal and parietal blood flow and glucose metabolism similar to that which is seen in AD (Kuhl *et al.*, 1984; Pizzolato *et al.*, 1988). In addition, it has been reported recently that diminished glucose metabolism has been demonstrated in the inferior frontal lobe of depressed PD patients (Mayberg *et al.*, 1990).

Thus, PD patients exhibit both generalized dementia and more specific frontal lobe behavioural disturbances. Physiological imaging studies have also demonstrated reduced frontal lobe perfusion and metabolism, as well as the temporoparietal hypometabolism and hypoperfusion which has been seen in AD. While it appears reasonable to suggest that these functional imaging patterns and patterns of cognitive performance may be related, no evidence yet exists to confirm this suspicion. We utilized SPECT scanning with a tracer technique to study relative rCBF in order to determine relationships between relative rCBF and cognitive function in PD, with the specific prediction that different patterns of cognitive decline would be related to decreased perfusion of different brain regions.

METHODS

Patients

We selected subjects from three groups: patients with PD, patients with AD, and healthy control subjects. Patients with PD were drawn from a neurology clinic at a Veterans Affairs Medical Center as well as from the community through referrals from physicians and support groups. Patients with AD were recruited from a university dementia clinic. Control subjects were spouses of patients and also were recruited from the community at large. Procedures for obtaining informed consent were approved by the Institutional Review Boards of all participating institutions.

All subjects were screened to exclude the following conditions: serious medical illnesses (including hypertension and diabetes), history of stroke, neurosurgical intervention (including thalamotomy) or any other neurologic illness, and premorbid history of major psychiatric illness (including depression). Neither the AD patients nor the control subjects were taking any medications during the period of the study, and the PD patients were taking only anti-parkinsonian medications as described below.

We studied 20 patients with idiopathic PD. Based upon the medical history and a brief cognitive screening examination, none of these patients was demented, nor did any show evidence of severe intellectual disturbance. Most were not seriously impaired in daily function, and in those who were, disability was clearly related to motor function, as opposed to cognitive ability. All patients underwent neurological examination by one of the investigators (W.J.J.) using the Unified Rating Scale for Parkinsonism (Koller, 1987). Overall disease severity was assessed with the Hoehn and Yahr scale (Hoehn and Yahr, 1967). Seven patients were stage II, 10 were stage III, and three were stage IV. All had evidence of bradykinesia, while the presence of tremor and rigidity was more variable. Eighteen of the patients were taking levodopa/carbidopa, while two were mildly affected and had not yet been started on medication. The mean duration of treatment with levodopa was 5.2 yrs (range 0–15 yrs). Six patients were also taking anticholinergic medications. All patients on levodopa had been adjusted to an optimal dosage and showed clinical benefit from treatment. Drug dosages were not altered during the time of the study.

Twenty-one patients who met current research criteria for probable or possible AD (McKhann *et al.*, 1984) were also studied. These subjects were selected from a pool of 50 AD patients who had undergone the identical neurological and cognitive evaluation and who met all the exclusionary criteria defined above. All subjects underwent a battery of laboratory tests and CT or MRI scans to ensure that cognitive dysfunction could be attributed to no other illness. The AD patients selected were matched with the PD patients for age and were mildly demented. By selecting patients with scores on the Mini-Mental Status Examination (MMSE) (Folstein *et al.*, 1975) of 19 or greater, we produced a group of 21 AD patients that was matched for age with the PD patients.

A total of 24 control subjects were studied. All subjects had a medical interview and examination to ensure that they were healthy and not suffering from cognitive complaints and met the criteria for study entry. Sixteen controls had SPECT scans, while eight underwent the neuropsychological testing described below. No control subject had both imaging and cognitive testing.

Neuropsychological function

All PD patients, AD patients and the eight neuropsychological controls underwent the identical battery of tests.

Neuropsychological tests were chosen with the goals of using measures that could be administered to a wide range of cognitively intact and impaired patients, and of providing a comprehensive assessment with an emphasis on frontal lobe function. Timed measures were not avoided entirely, but wherever possible we used scores that minimized the effect of speed. The battery of tests included the MMSE, the digit span subtest of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981), a shortened version of the California Verbal Learning Test (CVLTm) (Delis *et al.*, 1987), Delayed Recognition Span Test (DRST) (Moss *et al.*, 1986), Controlled Oral Word Association Test (COWAT) (Benton and Hamsher, 1983), drawings of a house and clock to command and copy, and a series of mental control items. Tests were administered to patients generally 2 h after they had taken their levodopa medication.

Depression was measured with a shortened version of the Geriatric Depression Scale (GDS) (Sheikh and Yesavage, 1986). The original GDS was developed specifically for use with elderly patients and was shown to have high sensitivity and specificity in normal and mixed psychiatric elderly samples (Yesavage *et al.*, 1983). The short form correlates highly with the original and consists of 15 brief yes/no questions asked of the patient. This instrument was selected as an appropriate measure of depression for parkinsonian patients because it has only a single item which directly measures somatic function (low energy) and because it has been shown to be a valid indicator of depression even in medically ill elderly patients (Norris *et al.*, 1987).

The mental control series is a modification and expansion of the mental control portion of the original Wechsler Memory Scale (Wechsler and Stone, 1945). The subject is given five familiar sequences (e.g. days of the week, counting) to recite, first forwards, then in reverse. The score is the percentage of the elements of each sequence that are recited in the correct order. The line drawings were scored on four five point scales; overall recognizability, perseveration, overdrawing and stimulus boundedness (Ober *et al.*, 1991). The overall recognizability ratings were made on the basis of the presence or absence of specified elements and did not penalize subjects for slowness, or for inaccuracies that might result from tremor or micrographia. Two raters scored the drawings independently, and a consensus was made when disagreements occurred.

Neuropsychological data were reduced to four cluster scores intended to assess specific cognitive functions, and an overall summary score. The specific cognitive functions for which cluster scores were constructed included memory, visuoconstructive ability, frontal lobe function and perseveration. These clusters were formed by selecting from the neuropsychological test battery scores that had a wide range of variability and minimal missing data, and rationally grouping them a priori by the cognitive functions they are assumed to measure. Each cluster was defined as the mean of the Z-transformed scores for each component measure.

The memory cluster was derived from the CVLTm total words recalled in five learning trials, long delay free recall, and total recognition memory score; the frontal lobe cluster included the COWAT, digit span backward, backward mental control score, DRST spatial span, and overdrawing ratings of house and clock drawings; visuoconstruction cluster included overall recognizability ratings of house and clock drawings; and the perseveration cluster was calculated by summing all perseveration scores for the COWAT, the DRST, and the house and clock drawings. For the memory, visuospatial and frontal cluster, a high number represents superior performance, while for the perseveration cluster a higher number represents more perseveration. The internal consistency for each cluster was evaluated using data from the group of 50 AD patients. Cronbach's α was calculated for each cluster, with coefficients of 0.54 for the frontal cluster, and 0.78 for both the memory and visuoconstruction clusters. A final summary score was calculated by summing all four clusters, after the perseveration score had been inverted.

Single photon emission computerized tomographic imaging

Single photon emission computerized tomographic imaging was performed within 1 mth of neuropsychological testing. Although medications were not discontinued for SPECT scans, all patients were studied approximately 2 h following the last dose of levodopa. We utilized a single-slice dedicated brain scanner with 12 crystal detectors and a resolution of 14 mm full width at half maximum in the plane of section and a slice thickness of 13 mm (Stoddart and Stoddart, 1979). The tracer [^{123}I]*N*-isopropyl-*p*-iodoamphetamine (IMP) was used to image relative rCBF. Approximately 185 MBq (5 mCi) of IMP was injected while the patient's eyes were open and ears unoccluded. Ten minutes after tracer injection scanning began at a level approximately 7 cm above and parallel to the orbitomeatal line. This level was selected by using a proportional system based upon the distance between the external auditory meatus and the top of the patient's head, as described by Matsui and Hirano (1978). The initial level was scanned for 20 min, following which the bed was advanced and a second level, 5 cm above and parallel to the orbitomeatal line was scanned for 20 min. If either image did not contain the usual anatomic landmarks, the slice was repeated immediately. Levels were selected to sample dorsolateral frontal and parietal cortex (level 1), orbitofrontal and temporal cortex (level 2) and occipital cortex (both levels). Level 1 passed through the mid-portion of the lateral ventricles, and level 2 passed through the basal ganglia.

Single photon emission computerized tomography data were analysed by drawing regions of interest in orbitofrontal and dorsolateral frontal cortex, temporal cortex, parietal cortex and occipital cortex. These regions were drawn by hand, by an operator trained in neuroanatomy, with reference to a standard anatomic computed tomography atlas (Matsui and Hirano, 1978). Activity densities for each region, in the form of counts/cm^3, were then computed. We then constructed an rCBF ratio, defined as the activity density in a specific region of interest normalized to the mean activity density of occipital cortex (from both hemispheres and at both tomographic levels) in the same patient. This rCBF ratio thus represents rCBF relative to rCBF in the occipital lobes.

Data analysis

Comparisons between groups were made using a multivariate analysis of variance (MANOVA). We performed separate MANOVAs for the results on the neuropsychological clusters and on the SPECT/rCBF ratios. Significance was determined by the Pillais Trace. If the results for the MANOVA were significant, *post hoc* Duncan New Multiple Range Tests were performed to ascertain which groups differed from each other for each dependent variable. Because this conservative statistic accounts for multiple comparisons following the omnibus test, we chose a level of α of 0.05.

As the primary aim of this project was the determination of the relationship between clinical features of PD, relative rCBF and cognitive function, we utilized stepwise multiple regressions with an α of 0.05 in order to investigate predictors of cognitive dysfunction in the PD group. As predictor variables, we entered age, disease duration, GDS score, years of education, MMSE score and the average of right and left dorsolateral frontal, parietal orbitofrontal and temporal rCBF ratios. As many studies have demonstrated that age is an important predictor of cognitive function in PD patients, we forced age into the regression equation as a predictor, while other variables were only incorporated into the model if they accounted for a significant ($P < 0.05$) proportion of the variance of the dependent variable.

RESULTS

Clinical features

A summary of the patient characteristics is shown in Table 1. Although the AD patients had significantly lower scores than PD patients and the controls on the MMSE, they

TABLE 1. SUBJECT CHARACTERISTICS

	AD	*PD*	*Neuropsych. controls*	*SPECT controls*
n	21	20	8	16
Age	71.1 (8.4)	68.0 (5.6)	66.5 (5.3)	65.6 (6.0)
Education	15.3 (3.6)	14.0 (4.3)	15.8 (2.2)	14.9 (3.0)
Sex (male/female)*	12/9	20/0	3/5	4/12
MMSE**	24.1 (3.3)	27.8 (2.0)	29.2 (1.2)	–
Disease duration***	3.0 (1.5)	7.3 (4.4)	–	–

Values in parentheses are one standard deviation, and all values of time are in years. *χ^2 $P = 0.0001$; **ANOVA showed significant group effect at $P = 0.0001$, with AD patients differing from PD patients and controls at $P < 0.01$; ***AD patients differed from PD patients by Student's *t* test $P = 0.0001$.

were generally quite mildly demented, with a mean score on the MMSE which is actually in the normal range for subjects of this age. Aside from one PD patient with a MMSE score of 23, all PD patients achieved scores of 25 or greater. The groups did not differ from each other on age or education. There were significant gender differences, however, as the PD group was composed entirely of men [$\chi^2(3) = 23.26$, d.f. 3, $P = 0.0001$], probably because the majority of patients were recruited from a veterans' hospital.

Within the PD group, patients taking anticholinergic drugs did not differ from patients who were not taking anticholinergic drugs on any neuropsychological cluster or any rCBF ratio.

Neuropsychological function

Patients' scores on the different neuropsychological clusters were, not surprisingly, intercorrelated. Table 2 shows the correlation matrix between the different clusters for the PD patients. The summary score was highly correlated with each individual cluster, undoubtedly because it was derived from the clusters. In addition, it was highly correlated with the MMSE score, an independent measure of overall cognitive function. Scores on the frontal cluster and the perseveration cluster were not highly correlated with the MMSE.

TABLE 2. INTERCORRELATIONS OF NEUROPSYCHOLOGICAL VARIABLES IN THE PD PATIENTS

	MMSE	*Memory*	*Constructions*	*Frontal*	*Perseveration*	*Summary*
MMSE	1.00					
Memory	0.56	1.00				
Constructions	0.61	0.45	1.00			
Frontal	0.09	0.37	0.55	1.00		
Perseveration	0.02	−0.43	−0.11	−0.49	1.00	
Summary	0.54	0.77	0.84	0.75	−0.54	1.00

The performance of the AD patients, PD patients and controls on each neuropsychological cluster is seen in Fig. 1. The MANOVA indicated a significant overall effect ($P = 0.0001$). *Post hoc* tests revealed that PD patients performed significantly ($P < 0.05$) more poorly than controls on all measures. The PD patients were significantly different from the AD patients only on tests of memory and on the summary score, on which they scored better than the AD group. The AD patients performed more poorly than controls on all clusters.

The diminished performance of the PD patients generally cannot be attributed to impaired motor control or slowed responding. All the memory scores are based on non-timed verbal responses. As described above, the drawing ratings which make up the constructions cluster did not penalize slowness or tremor. Time is not a determinant of the perseveration cluster score. Only the COWAT and mental control items were timed. Inspection of the mental control variable revealed that only one PD patient might have done better with more time and this could not have made more than a 10% difference in his score.

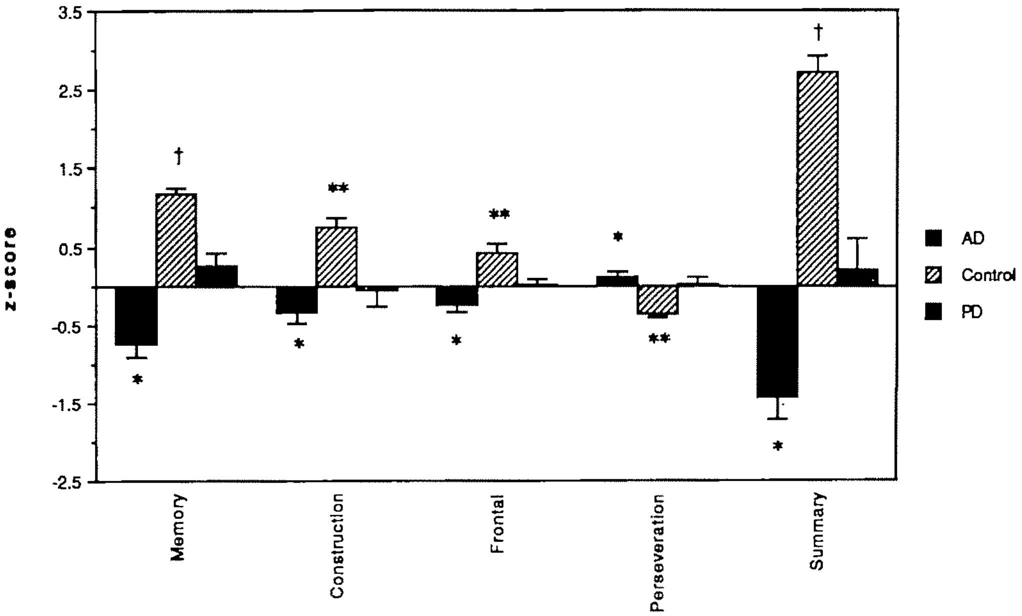

FIG. 1. Performance of the Alzheimer's disease (AD) and Parkinson's disease (PD) patients and control subjects on each neuropsychological cluster. Bars represent standard error of the mean. *Denotes AD patients significantly different from controls ($P < 0.05$); **denotes PD patients significantly different from controls ($P < 0.05$); †denotes PD patients significantly different from controls and AD ($P < 0.05$).

SPECT imaging

Examples of SPECT images obtained in an AD patient, a PD patient and a control subject are seen in Fig. 2. Figure 3 shows the results of the SPECT rCBF ratios for each brain region for all groups. The MANOVA showed a significant ($P = 0.0001$) overall group effect for the rCBF ratios. *Post hoc* tests showed that AD patients had significantly ($P < 0.05$) lower parietal rCBF ratios than controls in both hemispheres. While the PD patients had values intermediate between the AD and control groups in parietal cortex of both hemispheres, they were not significantly different from either in the right hemisphere but were significantly ($P < 0.05$) different from the AD patients (but not the controls) in the left hemisphere. In temporal cortex of both hemispheres, the AD patients showed significantly lower rCBF ratios than controls, and the PD patients were intermediate and significantly different from both the controls and AD patients. There were no between-group differences for orbitofrontal or dorsolateral frontal cortex for either hemisphere.

Because temporal cortical hypoperfusion is a marker for AD, we investigated whether PD patients with globally disturbed cognition had lower temporal rCBF ratios. We selected PD patients who scored in the lower 50th percentile on all four neuropsychological clusters. There were four such patients, whom we identified as the 'impaired PD group'. We then performed a MANOVA comparing these patients to the remaining 'unimpaired' PD group of 16 patients, as well as to the controls and AD group. Although

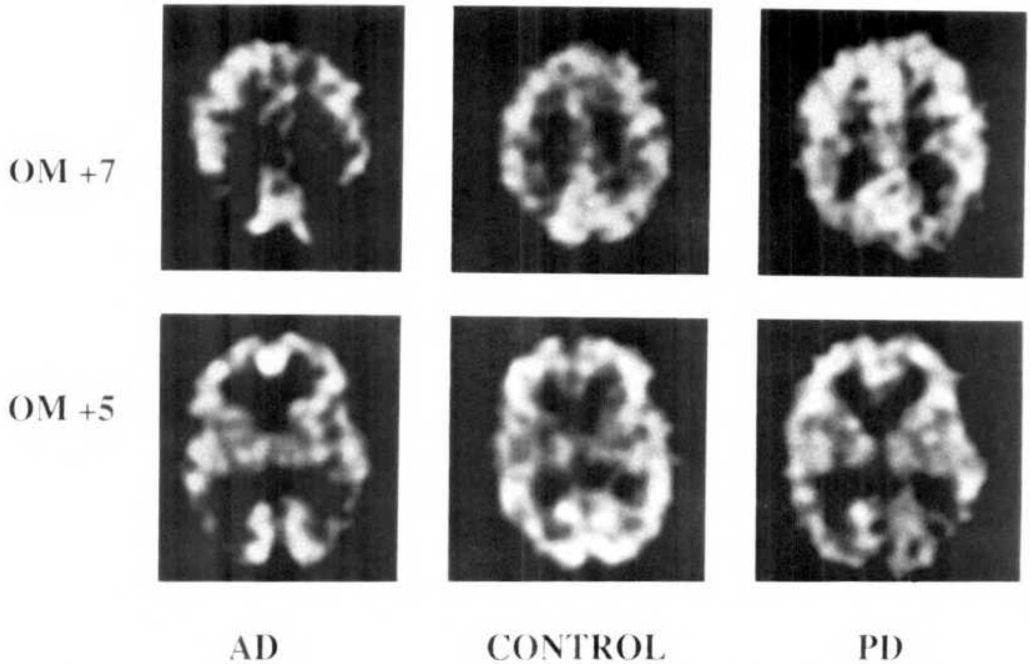

FIG. 2. Single photon emission computerized tomographic perfusion images from a patient with AD, a control subject, and patient with PD. Each patient has been scanned at two levels, 7 cm above the orbitomeatal line (OM + 7) and 5 cm above the orbitomeatal line (OM + 5). The AD patient demonstrates typical hypoperfusion in temporal and parietal cortex; this PD patient shows mild hypoperfusion in these regions.

the overall MANOVA was again significant ($P = 0.0001$), the only region in which impaired PD patients significantly differed from controls was in the temporal lobes. In left and right temporal cortex, the impaired PD patients showed significantly ($P < 0.05$) lower rCBF ratios than the controls, and were not different from the AD patients. In contrast, the unimpaired PD patients had significantly ($P < 0.05$) higher rCBF ratios than the AD patients in both hemispheres, and were significantly lower than the controls in the left hemisphere but not the right. These results are shown graphically in Fig. 4.

Relationships between cognitive function and rCBF ratios

Stepwise multiple regressions were performed using the predictor variables discussed in the data analysis section for the 20 PD patients. For each neuropsychological cluster, a different group of variables emerged as significant predictors of cognitive function. Results are shown in Table 3 for each neuropsychological cluster.

The strongest predictor of memory function was patient age, and the only other significant variable was disease duration. Despite the fact that age was forced into the prediction equation, it was a strong predictor, accounting for 42% of the variance in memory function. For construction performance, age was also a strong predictor, although adding the MMSE to the equation accounted for an even greater proportion of the variance

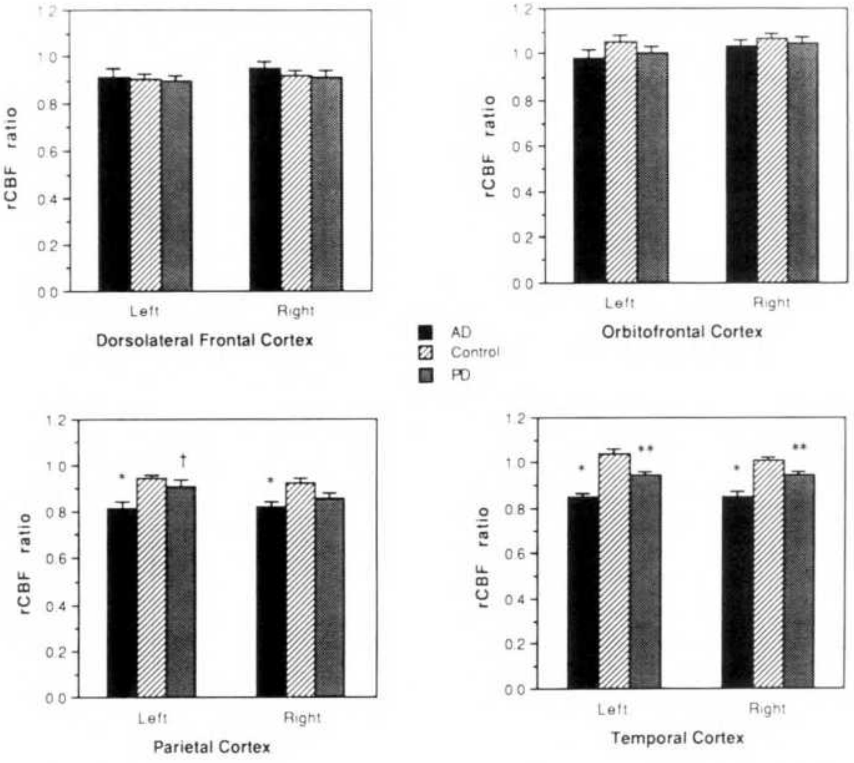

FIG. 3. Single photon emission computerized tomography/rCBF ratios for each cortical region as described in the text. Error bars represent SEM. *Denotes AD significantly different from controls ($P < 0.05$); †denotes PD significantly different from AD patients ($P < 0.05$); **denotes PD significantly different from both AD patients and controls ($P < 0.05$).

of the dependent variable. Although age accounted for 11% of the variance of the frontal lobe cluster score, both the score on the GDS and the dorsolateral frontal rCBF ratio added a greater proportion of the variance of this cluster. Similar results were seen for predictors of performance on the perseveration cluster, with the addition of educational level as a significant predictor. The regression equation for the cognitive summary score included a large number of variables with age as the strongest predictor, while the only rCBF ratio which was included was from the parietal cortex, which was a weak, but significant, predictor of overall cognitive function.

DISCUSSION

The findings of this study concern changes which occur in cognitive function and cerebral blood flow in PD, and their relationship to each other. Patients with PD showed decrements in multiple spheres of cognition, and had diminished temporal perfusion relative to controls. Furthermore, the patterns of relationships between relative rCBF and cognition differed for different cognitive functions, with temporal and parietal

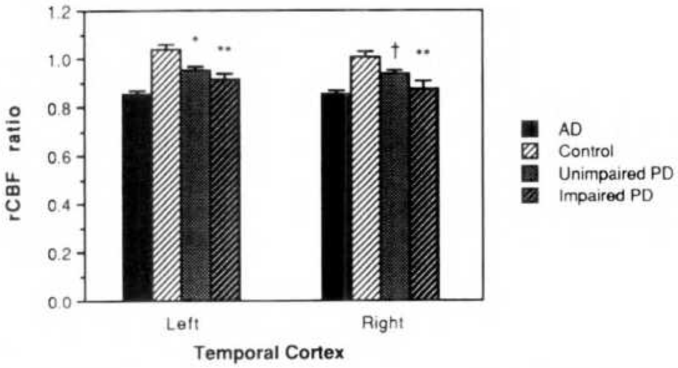

FIG. 4. Single photon emission computerized tomography/rCBF ratios for AD and PD patients. Patients with PD are defined as impaired or unimpaired as described in the text. Error bars represent SEM. *Denotes unimpaired PD patients different from AD patients and controls ($P < 0.05$); †denotes unimpaired PD patients different from AD patients ($P < 0.05$); **denotes impaired PD patients different from controls ($P < 0.05$).

TABLE 3. RESULTS OF STEPWISE MULTIPLE REGRESSION FOR THE PD PATIENTS

	R	ΔR^2	F	β
Memory cluster				
Age*	0.65	0.42	12.38	−0.048
Duration	0.85	0.30	20.75	−0.084
Construction cluster				
Age*	0.38	0.14	2.87	−0.016
MMSE	0.59	0.22	4.44	0.243
Frontal cluster				
Age*	0.34	0.11	2.29	−0.017
GDS	0.57	0.22	4.18	−0.084
Dorsolateral frontal rCBF ratio	0.73	0.21	6.15	1.795
Perseveration cluster				
Age*	0.31	0.09	1.87	0.030
Dorsolateral frontal rCBF ratio	0.55	0.21	3.72	−2.059
GDS	0.70	0.19	5.17	0.070
Education	0.78	0.13	6.01	−0.036
Summary score				
Age*	0.60	0.36	8.87	−0.103
GDS	0.72	0.15	7.90	−0.341
Education	0.82	0.17	9.84	0.246
MMSE	0.88	0.09	10.88	0.390
Parietal rCBF ratio	0.91	0.07	12.32	5.200
Disease duration	0.94	0.05	14.71	−0.111

Each dependent variable is listed, below which are the independent variables which were incorporated by the stepwise regression procedure, in the order in which they were incorporated to predict the variance of the dependent variable. *Denotes the fact that age was forced into the equation. Each value for R is cumulative, expressing the correlation coefficient for the regression equation which includes all predictors up to and including that step. ΔR^2 represents the additional variance accounted for in the dependent variable by incorporation of the new predictor. β represents the coefficient for each predictor in the final regression equation.

perfusion showing relationships to global cognitive performance and dorsolateral frontal perfusion showing relationships to tests of frontal lobe function.

Several methodological points are worthy of discussion. This study relied on clinically diagnosed patients without pathological verification, so that a proportion of patients, perhaps as great as 20%, may not have had idiopathic PD with Lewy bodies. This was hopefully minimized by the selection of only patients who had been responsive to levodopa therapy. Our use of the SPECT technique, as opposed to PET, has the disadvantage of being less quantitative. Patient positioning and region selection introduces an error due to variation in the placement of regions of interest, particularly in the z-axis, although this was minimized by the resolution of the scanner and our procedure for positioning subjects uniformly. The use of radioactive counts, with normalization in the form of a ratio of rCBF values, allows comparison between subjects but biases the results because the denominator, in this case occipital cortex, may be affected by the disease process under study. Previous studies, however, have shown small and non-significant decreases in occipital lobe rCBF in PD patients compared with controls (Wolfson *et al.*, 1985), which suggests that the biases in these ratios for the PD patients should be small. In AD, occipital cortex is also relatively unimpaired both pathologically (Brun and Englund, 1981) and metabolically (Duara *et al.*, 1986), which would also produce small biases in these ratios. Finally, the small degree of hypoperfusion expected in occipital cortex in both patient groups would tend to minimize differences between these groups and control subjects, and thus provide a more conservative test of our hypotheses. Another important issue is the possible effect of levodopa medication on our results. Although considerable data from animal and human studies show that levodopa increases rCBF, previous work demonstrated that these increases do not preferentially affect or spare frontal brain regions, with similar changes in rCBF in all cortical regions and in both PD patients and controls (Leenders *et al.*, 1985). These global increases in rCBF should not therefore differentially affect the regional ratio method we used to analyse our data.

We detected significant impairment in PD patients relative to controls in every sphere of intellectual function which we tested. On tests of constructions, frontal lobe function and perseveration, the PD patients performed at a level which was not significantly different from a group of mildly demented AD patients. The areas in which PD patients performed significantly better than the AD patients were memory, the summary score of global function and the MMSE, an independent measure of global cognitive ability.

It is important to note that despite the presence of these detectable cognitive deficits, the PD patients were not demented and were in moderate stages of the disease, thus comprising a similar group to that studied by Taylor *et al.* (1986*a*) and Gotham *et al.* (1988). The findings that non-demented PD patients performed in the AD range on measures of construction, frontal/executive function and perseveration probably reflects the fact that these functions are not severely abnormal in the early stages of AD, while they are mildly abnormal in non-demented parkinsonian subjects. The differences in memory and global cognitive function between the AD and PD groups are indicative of the severe and early involvement of memory function in AD and the pervasive nature of cognitive dysfunction which, by definition, occurs in dementia.

The SPECT indices of relative rCBF in the AD patients showed the expected decrements in temporal and parietal cortex which we (Jagust *et al.*, 1987) and others (Johnson *et al.*, 1987) have previously observed. The PD patients as a group did not demonstrate changes in frontal rCBF ratios, but did show diminished temporal lobe perfusion in both hemispheres which was intermediate between the controls and AD patients. While it is tempting to relate these intermediate rCBF ratio values in the temporal lobe to the intermediate performance on memory testing, temporal lobe rCBF ratios were not significant predictors of memory function in the PD patients. This is not surprising since the temporal lobe structures studied with SPECT imaging were neocortical, as opposed to the mesial archicortical regions more intimately involved in memory function. There did, however, appear to be an association between reduced temporal lobe rCBF ratios and global cognitive decline, since the cognitively impaired PD patients had temporal lobe rCBF ratios lower than controls and in the range of the AD patients. The only other rCBF ratio which was related to overall cognitive function was the parietal rCBF ratio, a weak predictor of the score on the summary cognitive scale.

These findings imply that temporal and parietal hypoperfusion are related to global cognitive dysfunction in PD. Because this perfusion pattern is also observed in patients with the dementia of AD, it is possible that the same pattern may indicate the existence of incipient AD in the PD patients. Work from other laboratories supports this view, as Kuhl *et al.* (1984) have noted diminished temporal and parietal regional cerebral glucose metabolism in demented PD patients. Pizzolato *et al.* (1988) also found diminished uptake of a different SPECT perfusion tracer ([^{99m}Tc]hexamethylpropyleneamine oxime) in parietal cortex of PD patients, with the degree of hypoperfusion related to the extent of mental deterioration.

In AD, temporoparietal hypoperfusion and hypometabolism have been suggested to reflect the predilection of these brain regions for the pathology of AD (Friedland *et al.*, 1985). Of particular relevance is the finding that these abnormalities are present early in the course of AD. We have found that 55% of AD patients in the earliest stages of the disease (when diagnosis is clinically difficult and memory loss is the only or the primary symptom) show temporal or parietal perfusion abnormalities (Reed *et al.*, 1989). Thus, the suggestion that these abnormalities might be caused by concurrent early AD in a group of PD patients without overt dementia is reasonable. Nevertheless, these perfusion abnormalities cannot be taken as definitive evidence of the presence of AD, since they have been seen in other dementias (Gemmell *et al.*, 1987).

Although temporal and parietal perfusion appear to bear a relationship to overall cognitive function in PD, other variables are stronger predictors of these abilities. In particular, age was related to every domain of cognitive performance and correlated most strongly with memory function and the summary score. This relationship between age and global cognitive function is consistent with other studies of PD patients which have demonstrated that older individuals are more likely to demonstrate cognitive decline and dementia (Hietanen and Teräväinen, 1988; Reid *et al.*, 1989). The mechanism of this association is unknown, but in light of our perfusion findings it is important to note that the prevalence of AD is also clearly related to age in the population at large (Evans *et al.*, 1989). The finding that disease duration was a predictor of both the performance on the memory cluster and the summary score, despite the fact that age was independently forced into the regression, also supports the view that disease duration is a significant determinant of cognitive decline in PD over and above the effect of age alone.

The variables which were associated with frontal lobe function, summarized by the frontal and perseveration clusters, showed a different pattern of relationships to regional brain function and clinical variables than that seen for tests of memory and global cognitive function. Both of these frontal lobe clusters were predicted by depression scores and dorsolateral frontal lobe perfusion in the stepwise regression model. The similar predictors of performance on these two clusters suggests that the two scales measure similar functions, or functions which are performed by similar brain regions. In addition, the finding that frontal perfusion, as opposed to temporal and parietal perfusion, is related to these neuropsychological measures is strong evidence that different pathophysiological mechanisms are responsible for diminished performance on frontal lobe tasks than for global cognitive decline.

These results confirm and extend the findings of others. Starkstein *et al.* (1989) have demonstrated that depressed PD patients perform more poorly than non-depressed PD patients on both global measures of cognitive function (the MMSE) and on tests of frontal lobe ability. We also found a relationship between depression and a measure of global cognitive function (the summary score) and between depression and performance on tests of frontal lobe function. Furthermore, these relationships were continuous, suggesting that even mild depression may contribute to cognitive decline, as was found by Mayeux *et al.* (1981) and Starkstein *et al.* (1989). The effect of depression on performance cannot be explained by either age or education, as these variables were separately incorporated into the regression equation and accounted for a smaller proportion of the variance of the frontal and perseveration clusters than did depression and dorsolateral frontal perfusion. While age acounted for a large proportion of the variance of the memory cluster and the summary score, it accounted for a much smaller proportion of the variance of the frontal and perseveration clusters.

We also found that dorsolateral frontal lobe perfusion was a determinant of performance on these measures of frontal lobe function. This bears some relationship to the findings of Mayberg *et al.* (1990) who used PET with [^{18}F]fluorodeoxyglucose to show that depressed PD patients demonstrate reduced glucose metabolism in orbitofrontal cortex compared with non-depressed PD patients and controls. This effect was also found to be continuous with regard to depression, with mild degrees of depression related to mild hypometabolism. Our findings are somewhat different, however, since they predominate in a different brain region (dorsolateral frontal rather than orbitofrontal), and they demonstrate that dorsolateral frontal perfusion is a determinant of cognitive function over and above the effect of depression because it was independently incorporated into the regression equation. The separate contributions of depression and dorsolateral frontal perfusion to performance on frontal lobe tasks suggests that they may produce their effects by different mechanisms, perhaps because the effects of depression on frontal lobe performance are mediated by a different brain region, that is, orbitofrontal cortex. This is also consistent with Alexander *et al.*'s (1986) description of two apparently distinct circuits linking the caudate nuclei to premotor cortex, defined as the 'dorsolateral prefrontal' circuit and the 'lateral orbitofrontal' circuit. While the lateral orbitofrontal circuit may thus be involved in the affective symptoms of PD, and may also contribute to the poor performance on frontal lobe tests, the dorsolateral circuit appears to be a separate factor in determining frontal lobe performance.

These results reflect current understanding of the relationships between frontal lobe function and cognition. Classical descriptions of the relationships between behaviour and frontal lobe function stress differences between the emotional and affective disturbances incurred with orbital lesions of the frontal lobes, and cognitive disturbances associated with dorsolateral lesions (Girgis, 1971). For example, patients with dorsolateral lesions perform poorly on tests of executive functions such as the Wisconsin Card Sorting Test, while those with orbital lesions do not (Milner and Petrides, 1984). Although perseveration is frequently associated with damage to orbitofrontal cortex, such behaviours may be a feature of damage in either brain region (Mesulam, 1986). Patients with PD, in particular, show deficiency in the performance of delayed response tasks (Freedman and Oscar-Berman, 1986), which has been suggested to be due to involvement of the dorsolateral prefrontal circuit (Alexander *et al.*, 1986). It is thus not surprising that our PD patients show a relationship between dorsolateral frontal perfusion and cognitive tests which reflect executive ability and perseveration.

The pathophysiological explanation for these changes cannot be obtained from our data since they do not indicate whether the disturbance in cognitive function and dorsolateral frontal perfusion are due to frontal lobe dopamine deficiency, or to diminished outflow from the caudate nucleus. Diminished caudate outflow would be expected to produce reduced frontal lobe metabolism, which would in turn cause diminished rCBF. Similarly, reduced dopamine in the frontal lobes might have direct effects upon both metabolism and rCBF. Because these studies were not performed in an activated state, however, the changes in perfusion are likely to represent a pathophysiological process present in even a resting condition and consequent to the degenerative process.

These data provide some clarification about the nature of cognitive loss in PD. The patterns of cognitive loss, relative rCBF and their relationship to each other suggest that different pathophysiological mechanisms may be responsible for generalized cognitive dysfunction and disturbed frontal lobe function. Furthermore, disturbances in frontal lobe function are complex, with both affective changes and frontal perfusion playing important roles. Although the results of this study do not define the aetiology of altered cognition in PD, they do demonstrate that different measures of cognition in PD are related to different physiological disturbances. Of particular importance is the notion that global cognitive decline and frontal lobe dysfunction are clinically and behaviourally distinct, related to dysfunction in different cortical regions and are likely to be produced by different physiological mechanisms.

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SIMPLE AND CHOICE REACTION TIME AND THE USE OF ADVANCE INFORMATION FOR MOTOR PREPARATION IN PARKINSON'S DISEASE

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SUMMARY

The effects of different types of advance information (warning signal, temporal cue, movement parameter cue), presented at different intervals before an imperative stimulus on reaction time (RT) were examined. Sixteen patients with Parkinson's disease and 16 age-matched normal controls performed a simple reaction time (SRT) task, an uncued, two partially cued (cueing of direction of movement or hand) and a fully cued visual four choice reaction time (CRT) tasks. An S1-S2 paradigm was used, where a warning signal/precue (S1) preceded the imperative signal (S2) by 0 (uncued and unwarned conditions), 200 ms, 800 ms, 1600 ms or 3200 ms. When the unwarned SRT and uncued CRT conditions were compared, the patients were slower than the controls in both conditions, with the group differences being larger for the CRT than the SRT task. Both patients and controls benefited from a warning signal presented before the imperative stimulus (S2), especially when it occurred 200 ms prior to S2. Advance information about the parameters of the required response was used by both patients and controls to preprogramme responses before the onset of S2. In both groups, RTs were generally faster for longer S1-S2 intervals. With an S1-S2 interval of 3200 ms, advance information about two movement parameters was 'fully' used by the patients to preprogramme responses such that their fully cued CRT was reduced to the level of SRT. In the controls, this process was complete by 800 ms. The most parsimonious explanation of the pattern of results across the SRT, fully cued CRT and uncued CRT conditions would be in terms of a slowness in response initiation in Parkinson's disease, which is a stage of processing common to all RT conditions. Deficits at one or more of the stages of processing unique to CRT are, however, necessary to explain the differentially greater slowness of uncued CRT in Parkinson's disease.

INTRODUCTION

The specific role of the basal ganglia in the control of movement is unclear. A current hypothesis is that the basal ganglia are normally responsible for 'the automatic execution of learned motor plans' (Marsden, 1982). This hypothesis has led to the study of preprogramming of movement in Parkinson's disease, the disorder considered to be most closely representative of the dysfunction of the basal ganglia and in particular the putamen (Marsden, 1982). Variations of reaction time (RT) paradigms have been employed to test this hypothesis. A number of studies have compared the simple RTs (SRTs) of patients with Parkinson's disease and controls (Heilman *et al.*, 1976; Yokochi *et al.*, 1985; Bloxham *et al.*, 1987; Viallet *et al.*, 1987; Montgomery and Nuessen,

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1990). In SRT tasks, the response is the same on every trial. The subject can, therefore, preprogramme the response which only needs to be initiated when the occurrence of the imperative stimulus is registered. Given that the stimulus is also invariant, identification and encoding of the stimulus are not necessary (Fig. 1*a*). In all these studies, the patients had significantly longer SRTs than controls. One interpretation of this finding is that patients with Parkinson's disease fail to preprogramme the responses before the onset of the stimulus. However, it is possible that prolongation of SRT in Parkinson's disease may result from deficits at one of the other two stages of processing preceding response execution in SRT tasks, i.e. stimulus registration and/or response initiation (Fig. 1*a*). As none of the features of the disease suggest any deficits in registration of the stimulus, an alternative explanation of the prolonged SRTs of patients with Parkinson's disease may be in terms of slowness in initiation of the response.

As response initiation is also one of the stages of processing involved in choice RT (CRT) tasks, this alternative explanation can be partly ruled out by demonstrating that patients with Parkinson's disease have a selective impairment on SRT but not CRT. A CRT condition is one in which a set of stimuli and responses are involved, each stimulus being associated with a particular response. In CRT (Fig. 1*b*), following presentation of the imperative stimulus, the particular stimulus presented needs to be identified, a response selected and programmed. Under conditions of low stimulus-response (S-R)

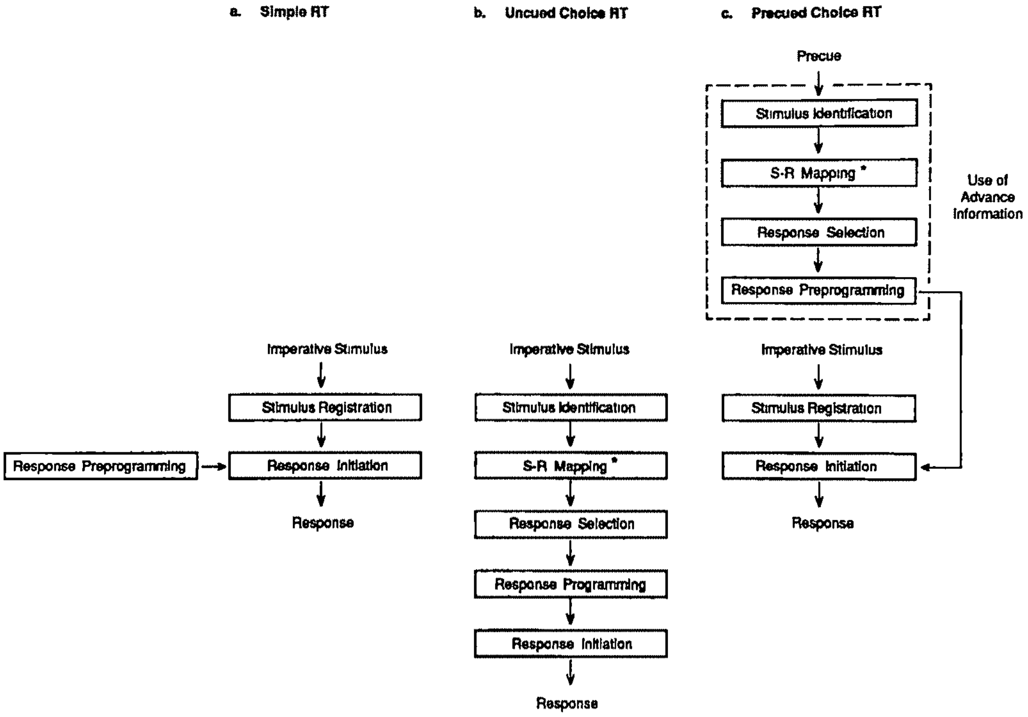

FIG. 1. The stages of processing involved in simple (*a*), uncued choice (*b*) and precued choice (*c*) RT conditions. *S-R mapping is only involved where the compatibility between the stimuli and responses is low.

compatibility, an additional stage of decoding S-R mapping rules will precede response selection. Consequently, a second group of studies have assessed SRT and CRT in Parkinson's disease compared with controls. Details of these studies are provided in Table 1. Their results have been contradictory. Some find that patients with Parkinson's disease are selectively or differentially more impaired on SRT, implying that they do not use advance information to programme motor responses (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Sheridan *et al.*, 1987; Goodrich *et al.*, 1989; Pullman *et al.*, 1988, 1990). In contrast, other studies report results that suggest that patients with Parkinson's disease can use advance information to preprogramme responses (Talland, 1963; Wiesendanger *et al.*, 1969; Girotti *et al.*, 1986; Stelmach *et al.*, 1986; Mayeux *et al.*, 1987; Lichter *et al.*, 1988; Reid *et al.*, 1989).

A 'true' SRT task is one in which the subject makes the same response to the same stimulus across trials (Fig. 1*a*). In a number of RT studies of Parkinson's disease (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Stelmach *et al.*, 1986; Lichter *et al.*, 1988; Pullman *et al.*, 1988, 1990), the SRT condition has in effect been a fully precued CRT task (Fig. 1*c*). In the latter task, a precue presented prior to the imperative stimulus provides complete information about the nature of the required response which allows preprogramming of the response (Fig. 1*c*). The fully precued CRT condition has been considered equivalent to SRT. However, this assumption is likely to hold only under certain conditions; for example, when subjects are given sufficient time to process the advance information.

The aim of the present study was to investigate the effect of two major factors that are likely to influence the pattern of SRT and CRT performance in patients with Parkinson's disease compared with age-matched controls. These are: (i) the type of advance information provided by a preparatory signal; (ii) the interval between the preparatory signal and imperative stimulus. The influence of these factors is of interest as it may partly account for the discrepant findings in the existing literature.

The type of information provided by a preparatory signal presented before an imperative stimulus can vary. A distinction can be made between warning signals, 'temporal' cues and 'movement parameter' cues. Any signal given a short time before an imperative stimulus will serve as a warning to the subject, allowing them to increase their level of alertness and readiness to respond. This facilitation appears to be optimal with a preparatory interval of 200 ms (Boff and Lincoln, 1988). In addition to a general alerting function, a preparatory signal may give more specific information about the forthcoming imperative stimulus. If presented at a fixed interval prior to the imperative stimulus on successive trials, the preparatory signal would operate as a 'temporal cue'. It allows the subject to prepare to respond at a particular time, and promotes the development of an anticipatory set or temporal expectancy. Finally, the signal may provide advance information about the nature of the response itself, e.g. it may inform the subject that he has to move to the upper key with his right hand when the imperative stimulus is presented. In this case it may be referred to as a 'movement parameter' cue. This information potentially allows the subject to preselect and preprogramme a specific response from a number of alternatives, provided there is adequate time between the cue and the imperative stimulus to take action. The amount of reduction of RT by 'temporal' and 'movement parameter' cues also depends on when they are presented relative to the imperative stimulus (Boff and Lincoln, 1988). It is important to note

TABLE 1 STUDIES COMPARING SIMPLE AND CHOICE REACTION TIME IN PARKINSON'S DISEASE

Author(s)	*PD group (n)*	*Control group (n)*	*SRT task*	*CRT task*	*Results*
Talland (1963)	25	25 age and sex matched	Unwarned and warned visual SRT, R = press key	Uncued 2 CRT	No differences between groups in any of the conditions. Eight severe PD slower than normals and mild PD
Wiesendanger *et al.* (1969)	17	10	Unwarned visual SRT	Uncued visual 2 CRT	Premotor RTs showed no group differences on either SRT or CRT. Combined premotor and motor RTs showed elongation of CRT but not SRT in PD
Evarts *et al.* (1981)	23	21 old and 11 young	Warned and precued visual 2 CRT. R = pronation or supination of a lever	Warned and uncued 2 CRT	No SRT/CRT data for controls provided but speculate that the difference in normals is greater than the 74 ms found in PD
Bloxham *et al.* (1984)	9	11	Precued visual 2 CRT. R = finger lifting	Warned 2 CRT	No differences between PD and controls on CRT but PD significantly slower on SRT
Girotti *et al* (1986)	11	21 age and sex matched	Warned and precued visual 3 CRT. R = lift finger from 'home' sensor and move to peripheral sensor	Warned 3 CRT to light stimuli	SRT and CRT differences between the patients tested 'on' and controls not significant
Stelmach *et al.* (1986)	8	8 age-matched	Warned fully cued visual 8 CRT R = lift finger from 'home' key and move to response key	Warned uncued or partially cued 8 CRT (i.e. 2 CRT, 4 CRT, 8 CRT)	SRT and CRT of PD significantly slower across all conditions
Sheridan *et al.* (1987)	Three groups of 6 male PD	Three groups of 6 age-matched males	Precued aimed visual CRT R = aimed movement of lever to move cursor into target	Uncued aimed CRT Control non-aimed RT task included	Independent groups rather than repeated measures design No differences between PD and controls on CRT, but PD significantly slower on SRT

Mayeux *et al.* (1987)	15	?12 or 15 age-matched hospital controls	Warned visual and auditory SRT	Warned 2 CRT	PD patients significantly slower than the controls on both SRT and CRT
Lichter *et al.* (1988)	39	38 age and sex matched	Precued visual 2 CRT. R = press button	Uncued visual 2 CRT	No differences between PD and controls on SRT, but PD significantly slower on CRT
Pullman *et al* (1988)	5 tested at high, mid, low L-dopa infusion levels	5 age-matched	Precued directional, visual 2 CRT R = flexion and extension wrist movements	Uncued 2 choice directional visual RT	No differences in CRT between normals and PD when tested 'on' levodopa. SRT significantly longer in PD than controls at all infusion levels
Reid *et al* (1989)	100, 72 early, 28 late-onset	50 age-matched	Warned simple visual RT. R = press key	Warned 2 choice visual RT	No differences between controls and whole PD group in SRT but CRT significantly elongated in PD
Goodrich *et al* (1989)	8 PD, 1 unmedicated	8 age-matched	Tactile SRT. R = lift finger	Tactile 2 CRT	No differences in CRT between normals and PD SRT significantly longer in PD
Pullman *et al.* (1990)	5 PD tested at high, mid, low L-dopa infusion levels	5 age-matched	Simple visual RT. R = 15 or 30 degrees wrist flexion	Two CRT. R same as SRT	Both SRT and CRT prolonged in the patients compared with normals. SRT and CRT differences significant for the normals but not patients. No effect of levodopa levels on either SRT or CRT

SRT = simple reaction time; CRT = choice reaction time (numeral is number of choices); R = response; PD = Parkinson's disease.

that the effect of both temporal cues and movement parameter cues includes the general warning effect in addition to that due to the specific information they contain.

The questions addressed in the present study were: (i) Is SRT selectively slowed relative to CRT in patients with Parkinson's disease compared with normals? (ii) Do patients with Parkinson's disease show the same pattern of RT facilitation as normals given the following different types of advance information: (*a*) use of a warning signal; (*b*) use of temporal predictability of the imperative stimulus; (*c*) use of advance information about the spatial location of a response?

METHODS

Design

A mixed between groups and within subjects design was used. Patients with Parkinson's disease and normal controls performed in each of the seven experimental conditions A to G listed in Table 2. Within each condition, a precue or warning signal (S1) preceded the imperative stimulus (S2) The S1-S2 interval was 0 ms, 200 ms, 800 ms, 1600 ms or 3200 ms

TABLE 2 THE EXPERIMENTAL CONDITIONS

Simple reaction time (SRT)	
A	Random block SRT
B	Fixed block SRT
Four choice reaction time (CRT)	
C	Uncued four CRT
D	Partially cued four CRT: cueing of hand
E	Partially cued four CRT: cueing of direction
F	Fully cued four CRT
Random block SRT	
G	Retest

Subjects

Sixteen patients with a clinical diagnosis of idiopathic Parkinson's disease and 16 age-matched normal volunteers with no history of neurological illness or head injury took part in the study. The details of the two groups are presented in Table 3.

The severity of Parkinson's disease was rated by an independent neurologist on the Webster symptom severity and clinical disability scale (Webster, 1968), who also classified the patients in terms of stage of illness using the Hoehn and Yahr (1967) criteria. The majority of the patients were in the mid stage of the disease with moderate symptoms (Table 3). All of the patients were on dopaminergic medication (average levodopa dose 643.3 mg, SD = 250.6 mg, with a selective peripheral decarboxylase inhibitor), and were tested while on their normal dosage of medication. In addition, five patients were taking anticholinergics, six were also on a monoamineoxidase B inhibitor (selegeline/deprenyl), and three were taking amantadine.

The two groups were matched in terms of age ($t = 0.13$, d.f. = 29, $P = 0.89$), sex ratio ($\chi^2 = 3.1$, d.f. = 1, $P = 0.08$) and handedness ($\chi^2 = 0.21$, d.f. = 1, $P = 0.65$). Screening for dementia was based on the Mini Mental Examination scores (MMS, Folstein *et al.*, 1975). None of the patients or controls scored below 25 on the MMS. Depression was assessed using the Beck Depression Inventory (BDI, Beck *et al.*, 1961). Two of the patients (12.5%) and one of the controls (6.3%) scored in the range 18 or above, indicating moderate or marked depression (Beck *et al.*, 1961). There were no differences in the

TABLE 3 DETAILS OF THE PARKINSON'S DISEASE AND NORMAL CONTROL GROUPS

	Parkinson's	*Controls*
Sex		
Male	10	5
Female	6	11
Hand		
Right	12	14
Left	4	2
Age (yrs)	63.7 (8.3)	64.1 (10.4)
Mini-mental scores	28.7 (1.4)	28.3 (1.6)
Depression	9.3 (5.7)	8.6 (6.9)
Age of onset (yrs)	53.9 (9.7) (range 34–73)	
Duration of illness (yrs)	9.8 (5.6) (range 1–20)	
Hoehn and Yahr (1967)		
I	1	
II	5	
III	9	
IV	1	
Webster rating	10.2 (4.7)	

mean BDI (t = 0.28, d.f. = 30, P = 0.78) and MMS (t = 0.82, d.f. = 30, P = 0.42) scores of the patients and controls.

One of the patients with Parkinson's disease had outlying RT values in most conditions. To keep the patient group homogeneous, this patient's data were eliminated. All analyses are based on the remaining 15 patients.

Reaction time tasks

The response apparatus comprised a box bearing six circular buttons each 1 inch in diameter. The two central buttons were black and were placed 6 in apart (distance from centre to centre), one on the left and one on the right. These served as the 'home' keys. Four inches above the 'home' keys were two red buttons, and the same distance below, two green buttons. The diameter of the four coloured response buttons were the same as the 'home' keys.

The general stimulus display procedure is presented in Fig. 2*a*. A variation of Rosenbaum's (1980) movement precueing technique with high S-R compatibility was used. The subject sat in front of a visual display unit (VDU), and was instructed to press and hold down one or both of the black 'home' keys with the index finger of the right, left or both hands, depending on the condition. This made a small cross appear in the middle of the screen, on which the subject fixated. After a variable delay (1–4 s), a precue/warning signal (S1) was presented. In all conditions, this signal was one or more empty squares. The imperative stimulus (S2) was the filling (becoming white on the black VDU background, which was virtually instantaneous) of the relevant square after an experimentally manipulated interval of 0 ms, 200 ms, 800 ms, 1600 ms or 3200 ms.

Examples from each experimental condition are depicted in Fig. 2*b*. The warning signal/precue was either superimposed on the fixation point (SRT conditions) or was presented in different spatial locations relative to the fixation point (CRT conditions). Depending on the experimental condition, the empty square constituted a warning signal only, a temporal cue or a movement parameter cue. When operating as a movement parameter cue, the spatial location of the empty square(s) relative to the fixation point provided

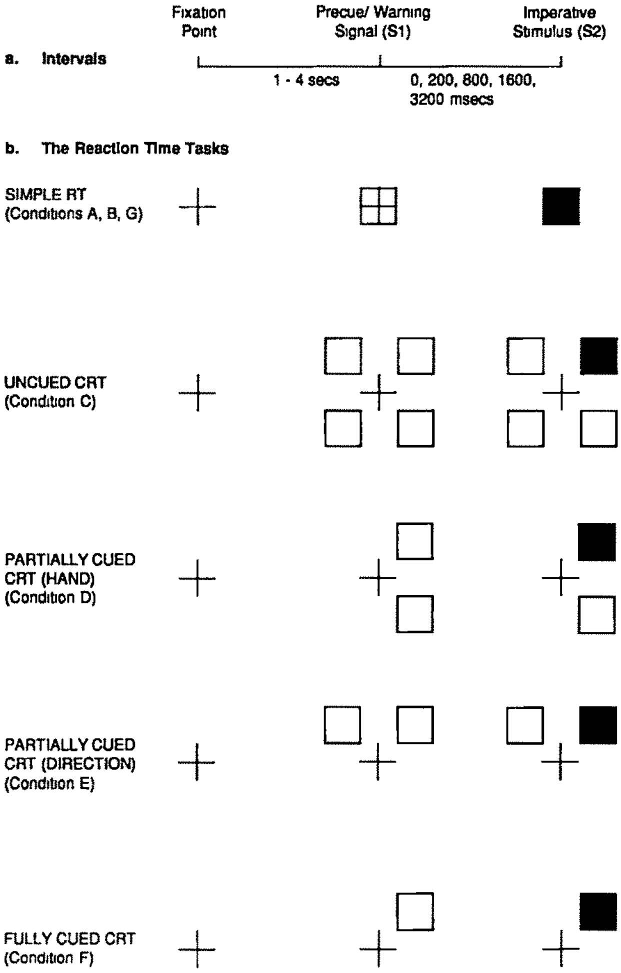
Fixation Point
Precue/ Warning Signal (S1)
Imperative Stimulus (S2)
a. Intervals
1 - 4 secs
0, 200, 800, 1600, 3200 msecs
b. The Reaction Time Tasks
SIMPLE RT (Conditions A, B, G)
UNCUED CRT (Condition C)
PARTIALLY CUED CRT (HAND) (Condition D)
PARTIALLY CUED CRT (DIRECTION) (Condition E)
FULLY CUED CRT (Condition F)

FIG 2. The intervals between appearance of the fixation point, the precue/warning signal and the imperative stimulus are shown in (*a*). The stimulus display in the various experimental conditions is presented in (*b*).

either partial information (hand or direction of movement) or complete information (both hand and direction specified in advance) about the impending movement in different experimental conditions. The subject was instructed to use the information conveyed by the precue to partially or fully prepare the movement before presentation of the imperative stimulus. The subject's task was to respond as quickly as possible to the imperative stimulus by releasing the 'home' key and moving to and pressing the relevant response key with the index finger. In each experimental condition, only the relevant keys were exposed. The screen cleared 500 ms after a response was made, and the next trial was started when the subject depressed the 'home' key. At its maximum, the screen configuration subtended a visual angle of about 0.2 degrees.

To discourage anticipatory responding, subjects were told to respond only after the presentation of the imperative stimulus. Three types of invalid trials were recorded: anticipations (reaction time less than or equal to 100 ms); long responses (reaction time greater than 3 s); decision errors (wrong response key pressed in CRT conditions). Reaction time and movement time measured from these trials were eliminated.

Trials were presented until the required number of valid trials (50 per hand in each SRT condition, 75 for both hands per CRT condition) were obtained. Reaction time (RT) was measured as the time between presentation of the imperative stimulus and release of the 'home' key. Movement time (MT) was the time taken to move from the 'home' key to a response key.

Experimental conditions (see Fig. 2*b)*

Simple reaction time

Random block SRT (Condition A). In this condition, each block of trials involved the same response (for example, moving right index finger from 'home' key to 'response' key) to the same stimulus. Half of the subjects in each group moved from the 'home' key to an upper response key while the other half moved from the 'home' key to a lower response key. Each subject performed two blocks of 50 trials (10 trials per interval), one block with each hand. In each group, the order of left- and right-hand performance was counterbalanced across subjects. The S1-S2 intervals were randomized.

Fixed block SRT (Condition B). This condition was identical to the random block SRT Condition A, except that the trials for each hand were organized into five sets of 10 consecutive trials for each of the S1-S2 intervals. Within each group, the order of intervals and the order of performance with the right and left hands was counterbalanced. As before, half of the subjects in each group moved from the 'home' key to an upper response key, while the other half moved from a 'home' key to a lower response key.

Four choice reaction time

The two movement parameters involved were hand (right versus left) and direction (up versus down). In four conditions, the response was either fully cued for hand and direction, partially cued for one of the two parameters or uncued. Each condition comprised 75 trials with 15 trials of each of the five S1-S2 intervals randomly mixed. An equal and randomly mixed number of right- and left-hand responses were incorporated.

Uncued CRT (Condition C). In the uncued block, four empty squares appeared on the VDU to the left and right and above and below the fixation point serving as a warning signal. After the S1-S2 interval, one of the four squares filled, indicating the movement to be made.

Partially precued CRT (Conditions D and E). In the partially cued trials, the subject was provided with partial information about the required movement In Condition D, the hand of response (right or left) was precued. Two empty squares appeared above and below the fixation point either to its left or right. After the S1-S2 interval, either the upper or lower square filled, and acted as the imperative stimulus. In Condition E, the direction of response (up or down) was precued. Two empty squares appeared to the left and right of the fixation point either above or below it. After the S1-S2 interval, the right or left square filled and indicated the exact response to be made.

Fully precued CRT (Condition F). In the fully cued condition, an empty square appeared on the VDU in one of the four possible positions above or below, to the left or right of the fixation point. After the S1-S2 interval, the square filled, instructing the subject to move to the appropriate response key. In this condition, the subject knew the precise nature of the movement to be made prior to the presentation of the imperative stimulus.

Order of testing

All subjects performed the SRT conditions first, followed by the four CRT conditions. This order allowed the subject to start with the conceptually simpler SRT task and sequential adding of new movement parameters from the SRT to the CRT conditions. The order of the four CRT conditions was counterbalanced across subjects. To assess possible fatigue/practice effects, the random block SRT condition was repeated at the end of the session.

RESULTS

Although RTs with the right hand (dominant in most cases) were in general faster than those with the left hand for both the patients and controls, the main effects or interactions relating to the use of the right or left hand were not significant. Therefore, the average of the right and left hand RTs was used in subsequent analyses.

Prior to addressing the major question, changes in SRT as a result of practice or fatigue were assessed by comparing the random block SRT conditions performed at the beginning (Condition A) and end (Condition G) of the session. For the patients, mean SRT across the five S1-S2 intervals at the beginning of the session was 430 ms compared with 458 ms at the end, an increase of 28 ms. For the controls, the respective values were 394 ms and 410 ms, with a 16 ms difference. There was no difference between the change in RT in the two groups (t = 0.88, d.f. = 29, P = 0.39).

Group differences in SRT versus CRT

To analyse differences between the patients and controls in 'true' (i.e. unwarned) SRT and CRT, data from the trials without a precue/warning signal (i.e. S1-S2 interval of 0 ms) were compared for the random block SRT task (Condition A) and the uncued CRT task (Condition C). A repeated measures analysis of variance (ANOVA) was performed with Group (Parkinson's versus controls) as the between subjects variable. The mean RTs of the patients and the controls for these conditions are shown in Fig. 3. The patients were slower than the controls across the two conditions [F(1,29) = 10.1, P = 0.003]. The main effect of Condition [F(1,29) = 84.5, P = 0.001] and the Group by Condition interaction [F(1,29) = 5.8, P = 0.02] were also significant. The patients were significantly slower than controls for both SRT (t = 2.1, d.f. = 29, P = 0.45) and CRT (t = 3.6, d.f. = 29, P = 0.001). The mean difference between SRT and CRT was significantly greater (t = 2.4, d.f. = 29, P = 0.02) for the patients (mean = 148 ms, SD = 71 ms) than the controls (mean = 86 ms, SD = 70 ms).

The effect of advance information

The effect of a warning signal on RT

In the condition where the precue did not provide any prior information about the spatial location of the impending movement, it acted purely as a warning signal. This

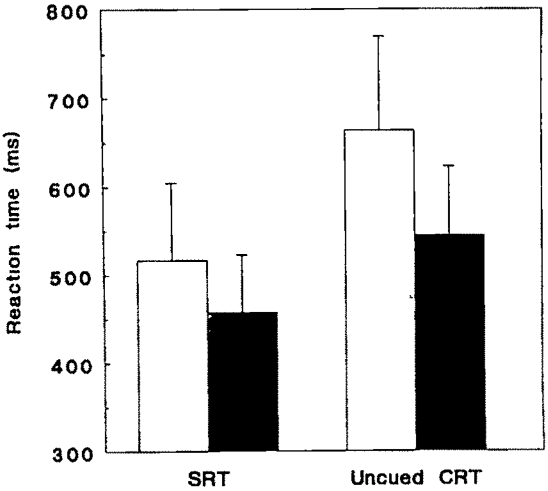

FIG. 3. The mean unwarned (S1-S2 interval of 0 ms) simple reaction time (SRT) and the unwarned and uncued choice reaction time (CRT) conditions of the patients with Parkinson's disease (in white) and the controls (in black)

was the case in the uncued CRT condition (Condition C). Although the precue also acted as a warning signal in the random block SRT condition, this condition differed from Condition C as the exact nature of the response was known to the subject in advance. For this reason the SRT condition was not included in this analysis. The relevant data for Condition C are presented in Fig. 4.

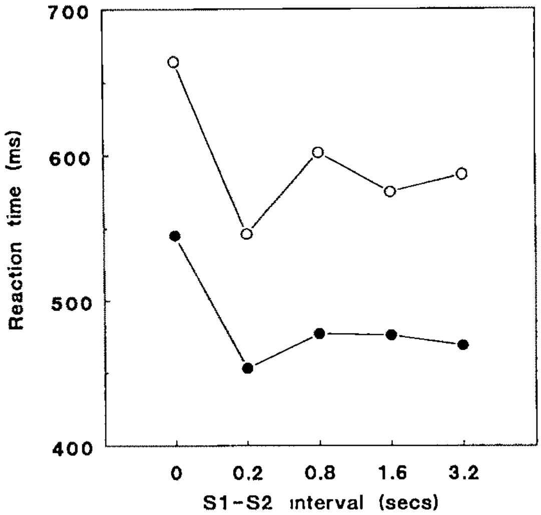

FIG. 4 The mean RTs of patients with Parkinson's disease (open circles) and controls (filled circles) across the five S1-S2 intervals in the uncued CRT condition.

A repeated measures ANOVA with Group (Parkinson's disease versus controls) and S1-S2 interval (0 versus mean of 200 ms to 3200 ms) was carried out on the data from the uncued CRT condition. To assess the general value of the warning signal, the RT trials where no preparatory signal was given (S1-S2 interval = 0 ms) were compared with the mean RT for trials of the other four S1-S2 intervals where a warning signal was present. The main effects of Group [F(1,29) = 13.9, P = 0.001] and warned versus unwarned trials [F(1,29) = 19.5, P = 0.001] were significant, whereas the Group by Trial type interaction was not [F(1,29) = 0.90, P = 0.35]. From Fig. 4, it can be seen that the presence of a warning signal speeded up RTs in both groups.

To determine whether RTs with the four warned S1-S2 intervals (200 ms, 800 ms, 1600 ms, 3200 ms) differed, a two-way repeated measures ANOVA was carried out with Group and Interval as the factors. The main effects of Group [F(1,29) = 14.5, P = 0.001] and Interval [Multivariate F(3,27) = 12.8, P = 0.001] were significant, whereas the Group by Interval interaction was not [Multivariate F(3,27) = 1.6, P = 0.21]. Given the differences between the four warning intervals, a number of *post hoc* comparisons were carried out to determine the S1-S2 interval at which the warning signal resulted in the greatest reduction of RT compared with the unwarned trials. Warned RT at each of the four S1-S2 intervals was significantly faster than the unwarned trials ($P < 0.01$). For both the patients and the controls, a warning signal 200 ms prior to the imperative stimulus speeded up RTs more than the other three S1-S2 intervals (Fig. 4).

The effect of temporal expectancy on SRT

In the fixed block SRT (Condition B), the precue was presented at a given S1-S2 interval for 10 consecutive trials. This potentially allowed the development of an anticipatory set or temporal expectancy of the imperative stimulus. Such an expectancy could not be developed in the random block SRT (Condition A). A repeated measures ANOVA with Group as the between subjects and Condition (random versus fixed block SRT) and Interval (0 ms, 200 ms, 800 ms, 1600 ms, 3200 ms) as the within subjects factors was performed to assess whether the effects of temporal expectancy were similar in the two groups. The relevant data are plotted in Fig. 5.

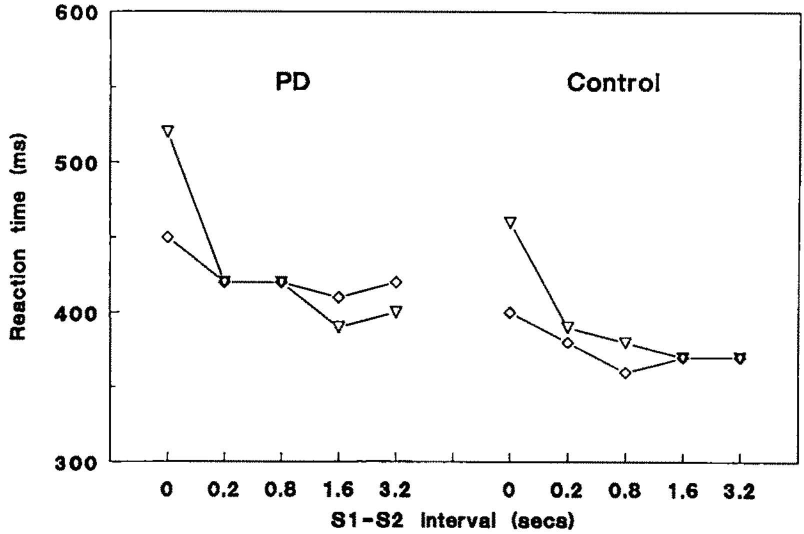

FIG. 5 The mean RTs of the patients with Parkinson's disease (PD) and the controls across the five S1-S2 intervals in the fixed block (diamonds) and random block (inverted triangles) SRT conditions.

The main effects of Group [F(1,29) = 4.5, $P = 0.04$], Condition [F(1,29) = 7.8, $P = 0.009$] and Interval [F(4,26) = 20.2, $P = 0.001$] were significant. Besides the Condition by Interval interaction [Multivariate F(4,26) = 12.4, $P = 0.001$] none of the other interactions were significant [Group by Condition: F(1,29) = 2.2, $P = 0.15$; Group by Interval: Multivariate F(4,26) = 1.7, $P = 0.18$; Group by Condition by Interval: Multivariate F(4,26) = 0.68, $P = 0.61$]. *Post hoc* analysis of the differences between the two SRT conditions at each of the five S1-S2 intervals revealed that, in both groups, the significant Condition by Interval interaction resulted from differences between the random and fixed block RT conditions at an S1-S2 interval of 0 delay [F(1,29) = 43.4, $P = 0.001$]; there were no differences between the two conditions at any of the other four intervals.

The use of advance 'movement parameter' information in CRT

The partial cueing of hand or direction. To assess whether any differences existed in the use of partial advance information about the arm to be used (left versus right) versus the direction of movement (up versus down), a repeated measures ANOVA was performed with Group, Condition (partial precueing of arm versus direction) and S1-S2 Interval as the factors. As expected, the main effects of Group [F(1,29) = 11.6, $P = 0.002$] and Interval [Multivariate F(3,27) = 8.5, $P = 0.001$] were significant. The Group by Interval interaction was not [Multivariate F(3,27) = 1.8, $P = 0.16$]. Neither the main effect of Condition [F(1,29) = 1.9, $P = 0.17$] nor any of its interactions were significant [Group by Condition: F(1,29) = 2.5, $P = 0.12$; Condition by Interval: Multivariate F(3,27) = 2.5, $P = 0.08$; Group by Condition by Interval: Multivariate F(3,27) = 0.49, $P = 0.69$]. This demonstrated that the use of partial advance information about the arm or direction of movement did not differ in either of the two groups or across the S1-S2 intervals. In subsequent analyses the data from Conditions D and E were collapsed and the means were used to represent partially cued CRT.

The effect of partial and full spatial information relative to no information in CRT. To assess whether advance information about the hand to be used and/or the direction of movement speeded up RT to the same extent in the two groups, a three-way repeated measures ANOVA was carried out, with Group, Condition (uncued Condition C, mean of partially cued Conditions D and E, fully cued Condition F) and Interval (200 ms, 800 ms, 1600 ms, 3200 ms) as the factors. The relevant data are shown in Fig. 6.

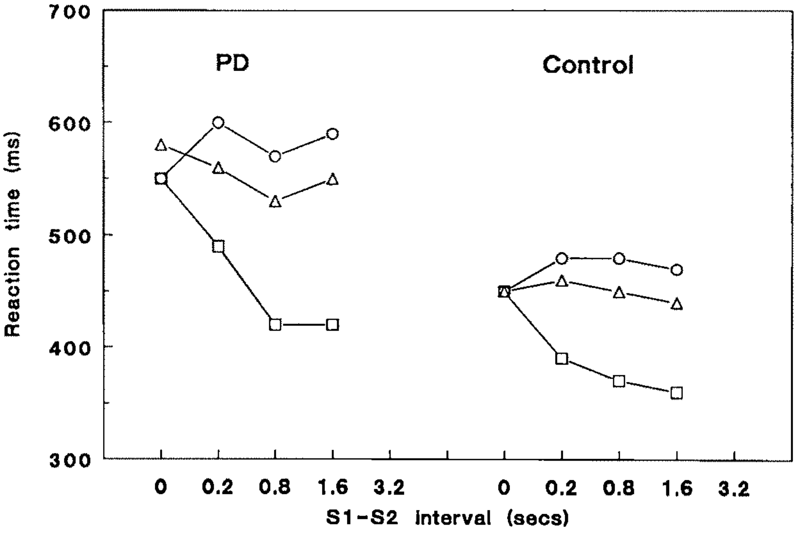

FIG. 6 The mean RTs of patients with Parkinson's disease (PD) and the controls across the 200 ms to 3200 ms S1-S2 intervals in the uncued (circles), mean of two partially cued (triangles) and fully cued (squares) CRT conditions.

The main effect of Group [F(1,29) = 13.1, $P = 0.001$], Condition [Multivariate F(3,28) = 57.8, $P = 0.001$] and Interval [Multivariate F(3,27) = 16.1, $P = 0.001$] were significant. The Group by Condition [Univariate F(2,58) = 2.8, $P = 0.07$] and the Group by Interval [Multivariate F(3,27) = 2.7, $P = 0.06$] interactions just failed to reach significance at the 5% level, whereas the three-way interaction was significant [Greenhouse-Geisser corrected F(4,111) = 3.2, $P < 0.05$]. The interaction between Condition and Interval was also significant [Multivariate F(6,24) = 21.8, $P = 0.001$].

To further analyse these interaction effects, a two-way (Group by Interval) ANOVA was carried out in each of the uncued, partially and fully cued conditions. As expected, the main effects of Group and Interval were significant in all three analyses. The Group by Interval interaction was not significant in the uncued [Multivariate F(3,27) = 1.6, $P = 0.21$] or the partially cued [Multivariate F(3,27) = 1.9, $P = 0.15$] CRT conditions. However, in the fully cued CRT condition, the Group by Interval interaction was significant [Multivariate F(3,27) = 3.9, $P = 0.02$]. This significant interaction in the fully precued CRT condition resulted from reductions of RT from the 800 ms to the 1600 ms S1-S2 interval being greater for the patients than for the controls ($t = 3.2$, d.f. = 29, $P = 0.003$).

The effect of full spatial information in CRT relative to SRT. An implicit assumption in many of the studies mentioned in the Introduction was that fully precued CRT is equivalent to SRT. These studies failed to determine the interval between the precue and imperative stimulus necessary for the precue information to be used and whether this interval was the same for the patients and controls. To examine these issues, a repeated measures ANOVA was performed with Group, Condition (SRT Condition A versus fully cued CRT Condition F) and Interval (200 ms, 800 ms, 1600 ms, 3200 ms) as the factors. The relevant data are plotted in Fig. 7. Data for the 0 ms S1-S2 interval are also shown in Fig. 7. The main effect of Group [F(1,29) = 6.5, $P = 0.02$], Condition [F(1,29) = 25.5, $P = 0.001$] and Interval [Multivariate F(3,27) = 39.0, $P = 0.001$] were all significant. The Group by Condition [F(1,29) = 9.2, $P = 0.005$], the Group by Interval [Multivariate F(3,27) = 5.7, $P = 0.004$] and the Condition by Interval

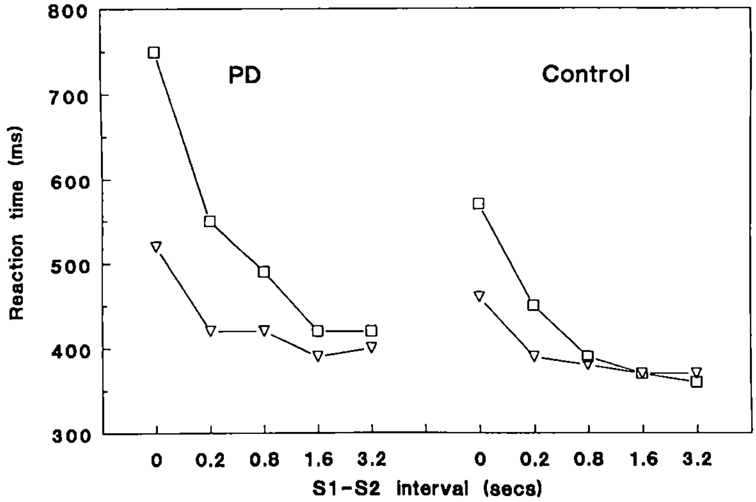

FIG 7. The mean RTs of the patients with Parkinson's disease (PD) and controls across the five S1-S2 intervals in the SRT (inverted triangles) and the fully cued CRT (squares) conditions.

[Multivariate F(3,27) = 16.1, $P = 0.001$] interactions were also significant. The three-way interaction just failed to reach significance [Multivariate F(3,27) = 2.7, $P = 0.06$].

The interactions were explored further by analysing the differences between the SRT and fully cued CRT at each of the four S1-S2 intervals within each group. In the Parkinson's disease group, fully cued CRT was greater than SRT at 200 ms ($t = 5.2$, d.f. = 14, $P = 0.001$), 800 ms ($t = 3.5$, d.f. = 14, $P = 0.004$) and 1600 ms ($t = 4.4$, d.f. = 14, $P = 0.001$) but not at 3200 ms ($t = 1.4$, d.f. = 14, $P = 0.18$). For the controls, fully cued CRT was slower than SRT only at the 200 ms S1-S2 interval ($t = 4.4$, d.f. = 15, $P = 0.001$), but there were no differences for 800 ms ($t = 0.86$, d.f. = 15, $P = 0.40$), 1600 ms ($t = 0.61$, d.f. = 15, $P = 0.55$) or 3200 ms ($t = 0.61$, d.f. = 15, $P = 0.92$).

Interpretation of these findings can be difficult given that the patients had a differentially longer CRT relative to SRT than the controls. To overcome this 'scaling' problem, the data were transformed. First, in each group difference scores between the fully cued CRT and SRT conditions were calculated for each S1-S2 interval. Secondly, the difference score at the 200 ms to 3200 ms S1-S2 intervals were rescaled using the 0 ms interval as the standard. For example, $r_{200} = (d_{200} - d_0)/d_0$, where r_{200} is the rescaled CRT/SRT difference score, d_{200} is the absolute difference score for 200 ms interval and d_0 is the absolute difference score for the 0 ms S1-S2 interval. The question of whether the S1-S2 interval at which fully cued CRT and SRT became equivalent was the same in the two groups was assessed by testing whether the rescaled difference score of the two groups differed significantly at each of the four intervals where advance information was provided. For the 200 ms interval, which is probably too short for the advance information in fully cued CRT to be used, the rescaled difference scores of the two groups were not significantly different ($t = 0.37$, d.f. = 28, $P = 0.72$). For the 800 ms S1-S2 interval, the group differences approached significance ($t = 1.9$, d.f. = 28, $P = 0.069$). For the 1600 ms interval, the rescaled difference scores of the patients and controls were significantly different ($t = 2.3$, d.f. = 28, $P = 0.03$). For the longest S1-S2 interval (3200 ms) the rescaled difference scores of the two groups did not differ ($t = 1.6$, d.f. = 28, $P = 0.12$). Therefore, rescaling of the data to take the differentially greater impairment of the patients on CRT into account did not alter the above pattern of results which suggested that the patients required a longer S1-S2 to use advance information and make their fully cued CRT equivalent to SRT.

Movement time

The mean MT of the patients and controls in the SRT and CRT conditions are shown in Table 4 for each S1-S2 interval separately. A repeated measures ANOVA was carried out on these data, with Group, Condition and Interval as the factors. As expected the MT of the patients was longer than that of controls across all conditions [F(1,29) = 11.7, $P = 0.002$]. The main effect of Condition [Multivariate F(4,26) = 6.9, $P = 0.001$] and Interval [Multivariate F(4,26) = 6.6, $P = 0.001$] were significant, but not the Group by Condition [Multivariate F(4,26) = 0.42, $P = 0.79$], the Condition by Interval [Multivariate F(14,16) = 0.74, $P = 0.72$], the Group by Interval [Multivariate F(4,26) = 0.39, $P = 0.81$] or the Group by Condition by Interval [Multivariate F(14,16) = 0.74, $P = 0.72$] interactions. The main effect of Condition arose from MTs

TABLE 4 MEAN AND STANDARD DEVIATION (IN PARENTHESES) OF MOVEMENT TIME FOR EACH OF THE S1-S2 INTERVALS IN THE SRT AND CRT CONDITIONS FOR THE PATIENTS AND CONTROLS THE UNITS ARE MILLISECONDS

		Parkinson's disease	*Controls*
Simple RT			
Interval	0	344 (126)	231 (52)
	200	347 (118)	227 (64)
	800	347 (126)	226 (59)
	1600	364 (127)	244 (76)
	3200	362 (136)	246 (63)
Four CRT			
Uncued			
Interval	0	428 (141)	291 (112)
	200	413 (131)	280 (91)
	800	415 (140)	298 (90)
	1600	449 (156)	295 (113)
	3200	447 (149)	312 (98)
Left/right cued			
Interval	0	433 (152)	274 (91)
	200	410 (141)	283 (108)
	800	430 (143)	292 (112)
	1600	423 (130)	296 (115)
	3200	435 (141)	305 (113)
Up/down cued			
Interval	0	472 (155)	311 (120)
	200	452 (121)	292 (115)
	800	437 (135)	291 (130)
	1600	443 (129)	308 (123)
	3200	455 (143)	297 (108)
Fully cued			
Interval	0	427 (154)	306 (125)
	200	419 (147)	278 (100)
	800	429 (151)	294 (122)
	1600	437 (145)	290 (108)
	3200	440 (145)	309 (124)

in the SRT condition being significantly faster than MTs in all the CRT conditions across the two groups ($P < 0.01$). There were no differences in MT among the four CRT conditions ($P > 0.05$). Across the conditions and groups, the significant main effect of Interval resulted from MTs in the 200 ms S1-S2 interval being faster than the 0 ms interval, and a gradual increase in MT from the 200 ms to 3200 ms S1-S2 intervals.

Errors

Table 5 shows the median number of each of the three types of errors, i.e. anticipations, long responses and decision errors. Very few errors were made in either group. A series of Mann-Whitney U tests were used to analyse group differences. The only difference between the groups was that the patients made more long responses in the uncued CRT condition than the controls (Mann-Whitney $Z = 2.1$, $P = 0.03$). Within each group, a series of Wilcoxon matched pairs tests were used to determine whether the number of errors made differed in conditions where advance information was or was not available (uncued CRT versus partially versus fully cued CRT). The only differences were in

TABLE 5 THE MEDIAN NUMBER OF ANTICIPATIONS (ANT.), LONG RESPONSES (LONG) AND DECISION ERRORS (DECISION) IN EACH OF THE CONDITIONS IN THE PARKINSON'S DISEASE AND CONTROL GROUPS

		Parkinson's disease			*Controls*		
		Ant	*Long*	*Decision*	*Ant.*	*Long*	*Decision*
Simple RT							
Random block	Median	0.0	0.0	–	0.0	0.5	–
	Minimum	0.0	0.0		0.0	0.0	–
	Maximum	1.5	3.0		0.5	3.0	–
Random block retest	Median	0.0	0.5	–	0.0	0.5	–
	Minimum	0.0	0.0		0.0	0.0	–
	Maximum	2.0	2.5		1.0	1.5	–
Fixed block	Median	0.0	0.50	–	0.0	0.75	–
	Minimum	0.0	0.0		0.0	0.0	–
	Maximum	1.0	7.5		0.0	3.5	–
Four CRT							
Uncued	Median	0.0	0.0	0.0	0.0	0.0	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	1.0	6.0	0.0	1.0	0.0	0.0
Arm cued	Median	0.0	0.0	0.0	0.0	0.0	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	7.0	3.0	7.0	1.0	3.0	2.0
Direction	Median	0.0	0.0	0.0	0.0	0.0	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	2.0	5.0	2.0	1.0	1.0	1.0
Fully cued	Median	1.0	0.0	0.0	1.0	0.0	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	6.0	4.0	1.0	4.0	2.0	1.0

terms of anticipation errors. Both the patients and controls made more ancipation errors in the conditions when they had full prior knowledge about the required movement (fully cued CRT) compared with when they did not have such information (Condition C versus F: patients: $Z = 2.9, P = 0.004$; controls: $Z = 2.8, P = 0.005$). In addition, compared with the uncued CRT condition, the patients made more anticipation errors when provided with partial information about either the direction of the impending movement ($Z = 2.2$, $P = 0.03$) or the hand to be used ($Z = 2.4$, $P = 0.02$).

Effects of severity of Parkinson's disease

Patients with a Hoehn and Yahr stage of illness ratings of I and II (n = 6) were compared with those with a stage of illness of III and IV (n = 9). In all the above comparisons, patients with more advanced Parkinson's disease had longer RTs than those who were at an earlier stage of illness. The difference in RT between the two subgroups was 52 ms for the SRT condition (mean across five intervals) and ranged from 81 ms to 103 ms to 134 ms for the uncued, partially cued and fully cued CRT conditions, respectively. However, none of the main effects or interactions relating to stage of illness were significant. The Webster ratings were not strongly associated with SRT or CRT or with MT. Correlations were mainly positive but small, ranging from 0.04 to 0.25. Duration of illness had positive and significant ($P < 0.01$) correlations with SRT (r = 0.67), and the four CRT conditions (Conditions C: r = 0.64; D: r = 0.65; E: r = 0.71; F: r = 0.78).

DISCUSSION

To summarize the main results: (i) compared with normals, patients with Parkinson's disease were slower in initiating and executing responses in both SRT and CRT conditions. (ii) The difference between the groups was greater for CRT than SRT. (iii) Patients and controls benefited equally from a warning signal especially when this was presented 200 ms prior to the imperative stimulus. (iv) The patients were able to use advance information about two movement parameters to prepare responses in advance of an imperative stimulus, but required longer than controls to make full use of that information.

Before the implications of the results for motor programming in Parkinson's disease are considered, three factors that could have potentially confounding results will be discussed. These factors are (i) fatigue effects in the course of the session; (ii) effects of precueing on MT; (iii) the pattern of errors across conditions.

As the introduction of new movement parameters in the CRT conditions could have influenced the strategies adopted in the SRT task, the latter condition was always performed first. Despite clinical reports suggesting that patients with Parkinson's disease fatigue more quickly, at least for a relatively undemanding motor task such as performance of a SRT condition, the fatigue effect observed in the present study by comparing mean SRT at the beginning (Condition A) and the end of the session (Condition G) was relatively small and similar for the patients and age-matched controls. Fatigue effects can, therefore, be excluded as a possible factor for the patients' differential impairment on the CRT tasks.

If the RT data are to be used to assess the programming of responses, it is important to show that manipulating the availability of advance information has no effect on MT. The MTs of the patients were slower across all conditions. In both groups, MTs in the SRT condition were faster than those in the CRT conditions. Provision of advance information had no effect on MTs in either group, as MTs did not differ across the four CRT conditions. This suggests that motor programming was relatively complete at the end of the RT period when the subject's finger lifted from the home key and before setting off towards a response key. The absence of an effect of precueing on movement execution processes allows inferences based on the valid premise that the influence of precueing is reflected in RT changes (Lépine *et al.*, 1989). The S1-S2 interval produced a significant effect on MT. In both groups and across all conditions, MT gradually *increased* across the 200 ms to 3200 ms S1-S2 intervals. This is in contrast to the significant effect of interval on RT which was due to a gradual *decrease* of RT across the 200 ms to 3200 ms intervals. This effect of S1-S2 interval on MT suggests that the speed of movement execution is reduced with longer intervals between the preparatory signal and the imperative stimulus, perhaps by alteration of the subject's general level of motor readiness. However, as noted above, this increase in MT was independent of the number of choices, presence/absence of advance information or group (patient versus control) membership.

Across the conditions, very few errors were made by either the patients with Parkinson's disease or the controls. In both groups the majority of errors were anticipations in the fully precued CRT condition. This is a further indication that the motor parameters which were precued in advance were being used by subjects to preprogramme the required response, and that anticipatory errors arose from initiation of the prepared response prior to presentation of the imperative stimulus. As the number of errors were low and as trials on which an error occurred were excluded, the RT findings are not in any way confounded by differential error patterns in the two groups.

In the following discussion, the results of the present study will be contrasted with the finding of a selective impairment in SRT in some previous studies which has been taken to imply that patients with Parkinson's disease are unable to use advance information to preprogramme responses. The important role played by the instructions to and strategies adopted by the patients will be discussed as a possible reason for the discrepancy between the results of the present and some previous studies.

Do patients with Parkinson's disease have deficits in the level of arousal or motor readiness?

As noted above, any form of signal preceding an imperative stimulus will warn the subjects and will allow them to increase their readiness to respond. In interpreting the effects of advance spatial (movement parameter) or temporal (temporal cue) information, it is important to know whether the patients show a normal response to a warning signal which only operates to alert them to the occurrence of the imperative stimulus and conveys no other information. This was indeed the case. A warning signal had a similar facilitatory effect on the CRTs of the patients and controls, with the reduction of CRT with a warning signal being maximal at 200 ms in both groups (Fig. 4). The effects of warning signals on SRT were not assessed because in this condition warning signal effects are confounded with information about the exact response which is known by the subject in advance. Nevertheless, in the SRT condition, warning stimuli presented prior to S2 also speeded RT in both groups. In the present study, the SRT and CRT of both the patients and controls were speeded up by warning signals. The results of Talland (1963), Heilman *et al.* (1976) and Bloxham *et al.* (1987) have also shown that the SRTs of patients with Parkinson's disease were reduced when a warning signal was included. Together these results suggest that the deficits in SRT and CRT in Parkinson's disease cannot be attributed to nonspecific disturbances of arousal. A similar conclusion was arrived at by Heilman *et al.* (1976).

An increase in response readiness can also be produced if a warning signal is given at a fixed interval before the imperative stimulus. Studies in normal subjects have shown that a constant preparatory interval (PI) speeds up RTs compared with a condition where the PI changes from trial to trial (Zahn and Rosenthal, 1966; Granjon *et al.*, 1973; Requin *et al.*, 1973). The present results, however, failed to replicate this finding in either group. This may have been due to the relatively small number of trials (10) at each fixed block of S1-S2 intervals. An unexpected difference between the fixed and random block SRT conditions was that RT for the unwarned interval (S1-S2 of 0 ms) was faster in the context of a fixed block of S1-S2 intervals than when the same condition was presented within a randomized block. This effect may be explained by different strategies in the two SRT tasks. In the randomized condition, the subject can expect a warning signal on 80% of trials and, therefore, may choose to wait for S1 before getting ready to respond. Consequently, when the warning signal does not occur, the subjects are slower to make a response. In contrast, when the subject knows that there will be no warning as in the fixed block condition, the best strategy is to maintain a general response readiness prior to S2. The choice and use of these strategies appeared to be the same in the patient and control groups.

Together, these results suggest that slowness of movement initiation in Parkinson's disease cannot be attributed simply to deficits in the level of arousal or motor readiness.

Do patients with Parkinson's disease have deficits in motor programming?

This question can be addressed from two aspects of the present data. First by examining the comparative differences between the patients and controls on the uncued and unwarned SRT and CRT tasks. Secondly, by assessing the use of advance movement parameter information in the two groups. Each of these will be considered.

Evidence from comparison of SRT and unwarned and uncued CRT. Compared with uncued CRT, the nature of the response is known in advance in SRT paradigms. The opportunity for preprogramming in the SRT condition is one factor accounting for the faster responses observed with SRT compared with CRT (*see* Fig. 1*a*,*b*). In Parkinson's disease a number of studies have found a selective impairment in SRT relative to CRT (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Sheridan *et al.*, 1987; Pullman *et al.*, 1988; Goodrich *et al.*, 1989). From this it has been inferred that the patients are failing to preprogramme their responses in the SRT condition, and that they are treating SRT as if it were CRT.

The present study failed to replicate this pattern of results. As in the other studies, the patients were impaired on SRT, but they showed an even greater impairment on the uncued CRT task. This result does not exclude the possibility that the patients were failing to preprogramme in the SRT condition as suggested by earlier studies. However, from the obtained pattern of SRT/CRT deficits, it is perhaps parsimonious to assume that slowing in both tasks was due to some stage common to the two tasks such as stimulus encoding or response initiation (Fig. 1*a*,*b*). Even assuming this, however, the patients still showed an additional slowing in the CRT task relative to SRT. This suggests a deficit in some stage of processing unique to the CRT task; for example, stimulus identification, S-R mapping or response selection (Fig. 1*b*).

Not all previous studies have found a selective SRT deficit in Parkinson's disease. Of the 13 studies in Table 1, those of Stelmach *et al.* (1986), Mayeux *et al.* (1987) and Pullman *et al.* (1990) found patients with Parkinson's disease to be significantly impaired on both SRT and CRT. In addition, the eight most severely affected patients in Talland's (1963) study obtained a pattern identical to the present results and showed a differentially greater impairment on CRT.

Evidence from the use of advance movement parameter information in CRT. For both the patients and controls, mean CRTs were longer when both hand and direction of movement remained to be specified (uncued Condition C), than when both parameters had been specified previously (fully cued Condition F). For S1-S2 intervals of 800 ms, 1600 ms and 3200 ms, this relative speeding up of CRT with provision of advance information was obtained for every patient and control subject. In addition, both for the patients and controls, provision of partial advance information about the hand or the direction of movement resulted in faster RTs relative to the uncued CRT task, but did not speed up RTs as much as the fully cued CRT condition. From these results it can be inferred that patients as well as controls made use of the precues to preprogramme responses. The same conclusion was arrived at by Stelmach *et al.* (1986) in their study of precued RTs in Parkinson's disease.

The conclusion that the advance movement parameter information provided in a precue speeds up RT because the programming of the response can occur prior to the imperative stimulus requires two assumptions to be made. The first assumption is that prior to presentation of the imperative stimulus, a 'prototypical programme' is constructed on the basis of the motor parameters that are common to all alternative responses. The CRT for any one response would include the time to add the information specific to that response to the prototypical programme. Another possibility to consider, however, is that with the limited number and types of simple responses used, fully specified motor programmes for each of the alternative responses might be prepared and then held in readiness in the motor buffer. In this case, the CRT would reflect the time taken to select the correct, preprogrammed response from the set of those assembled. Can these two alternatives be distinguished from the present data? In the present study, the fact that partial precueing of one movement parameter speeded up RT compared with the uncued CRT condition, but not to the same extent as the fully precued condition, provides some indirect support for the proposal that prototypical programmes are constructed.

The second assumption of the precueing RT paradigm is that the facilitation of RT by provision of advance information results from the preprogramming of responses. However, precueing might also influence two other stages of processing: stimulus evaluation and identification as well as response selection (Fig. 1*b*). The fact that precueing effects cannot be unambiguously attributed to response programming was noted by Stelmach and Larish (1981) and Goodman and Kelso (1980) in studies which showed that the effectiveness of precueing depended on S-R compatibility. In these studies, precueing effects were only obtained with incompatible S-R configurations, where it is possible that some of the effects of precueing were mediated by reducing the number of alternatives and hence facilitation of stimulus identification, S-R mapping and response selection. In later studies (Bonnet *et al.*, 1982), however, precueing effects have been obtained when the S-R compatibility was high, as was the case in the CRT conditions of this study. Therefore, the present results are less likely to be confounded by the nonmotor factors of stimulus identification and response selection and the precueing effect can be attriuted to facilitation of preprogramming.

If preprogramming is to take place, the individual will require sufficient time to identify the precue, map the spatial information onto the set of possible responses and then programme the response. It is not surprising, therefore, to find that the interval between the precue and the imperative stimulus also produced a major effect, with RTs being generally faster for longer S1-S2 intervals in both groups. The longer the interval, the more time is available to process the advance information and preprogramme the response. The present results confirm previous findings obtained for precued RTs of young normals using S1-S2 intervals ranging between 100 ms and 1000 ms (Miller, 1982) and from 475 ms to 3000 ms (Reeve and Proctor, 1984).

According to the additive factor method of Sternberg (1969), if two factors affect the same stage of processing their effects will be interactive, whereas when they operate on different stages of processing, their influence will be additive. The fact that the

'Condition by Interval' interaction was significant suggests that precueing of movement parameters and the interval between the precue and imperative stimulus both affect the same stage of processing which, we argue, is likely to be the programming of responses. In this context, the significant interactions obtained between Group, Condition and Interval have implications for understanding how advance information was processed across the 200 ms and 3200 ms intervals in the two groups. These will be considered next.

The present data revealed that the patients with Parkinson's disease required a longer S1-S2 interval to 'fully' process the advance information than the controls. The S1-S2 interval at which fully cued four CRT became the same as 'true' SRT was different for the patients and controls, even after rescaling the data to take the differentially greater CRT impairment of the patients into account. For the controls, advance movement parameter information reduced CRT to the level of SRT by 800 ms. For the patients with Parkinson's disease, fully cued CRT was the same as SRT only with an S1-S2 interval of 3200 ms, suggesting that a longer interval was required for use of advance movement parameter information to preprogramme responses. However, the Group by Interval interaction was significant for the fully cued CRT condition, where two movement parameters had to be preprogrammed. When only one parameter was known, as in the partially cued conditions, the Group by Interval interaction was *not* significant. This suggests that the presence/absence of a preprogramming deficit in Parkinson's disease may depend, in part, on the amount of information, as well as the time available to make use of it.

The slowness in use of advance information in Parkinson's disease at least when two movement parameters are available, may result from one of three different processes. First, it is possible that the patients start using the advance information at the same point in time as the controls but are slower in processing such information. The second possibility is that compared with the controls, there is a delay before the patients with Parkinson's disease start to process advance information. The third possibility is that at any point in time, the patients are less likely to make use of advance information to preprogramme a response. The control subjects appeared, on average, to have an almost 100% probability of using the information within 800 ms. If the probability of patients using the information within the same period was only 25%, for example, then it would take longer before the cumulative probability approached 100%.

In order to promote uniformity of preparatory strategies in the present study, all subjects were explicitly instructed to use the advance information to fully or partially prepare the response prior to the imperative stimulus. Evidence from young normals suggests that instructions to the subjects can influence the degree of preparation (Miller, 1982). The instructions to and the strategies adopted by the subjects are especially pertinent when studying patients with Parkinson's disease. The selective impairment of patients relative to normals on precued CRT (Fig. 1*c*) (taken as equivalent to SRT) but not uncued CRT (Fig. 1*b*) tasks in previous studies (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Sheridan *et al.*, 1987; Pullman *et al.*, 1988) may have resulted from a failure or inconsistency of the patients in using advance information and preprogramming of responses, as they were not explicitly instructed to do so. That patients with Parkinson's disease adopt a different strategy regarding preprogramming, at least initially, is suggested by a study of Worringham and Stelmach (1990), which showed differential effects of practice on SRT and CRT in patients and controls. Early in practice, the performance of the patients suggested that they were failing to preprogramme in SRT and were using the same strategy of post-stimulus motor preparation in both SRT and CRT tasks. With practice, however, SRT showed a differential improvement in the patients relative to the controls, a result consistent with a change of strategy and adoption of a preprogramming strategy.

As well as the use of explicit instructions, the precise nature of the precueing task itself may influence the probability of the subject preprogramming a response. As already noted, the compatibility of the stimulus and response may have an impact. A second factor affecting the probability of preprogramming may lie in whether the information provided by the cue is merely useful or essential for making the subsequent response. Sudevan and Taylor (1987) distinguish between two types of preparatory signal. A 'prime' is a signal which is useful to the subject but not essential, as the same information will be available in S2. In contrast, a 'cue' provides essential information which is not subsequently repeated in S2. With a cue, the subject must, therefore, attend to and process the information in S1 if they are to make the correct response. In the present study, as well as all previous investigations of Parkinson's disease with precued CRT, the advance information provided has been useful but not essential, i.e. in effect a prime. This methodology can render preprogramming of response prior to the imperative stimulus an optional strategy. The question of preprogramming strategy could be assessed in future studies by comparing primed and cued CRTs. If patients with Parkinson's disease are failing to preprogramme when this is optional, they should show a greater difference between primed and cued CRT than control subjects. This comparison could also clarify whether the inconsistency and/or slowness in motor programming in Parkinson's disease are compensatory strategies (Shallice, 1988), 'consciously' or 'unconsciously' adopted by the patients in adaptation to a 'noisy' (Bloxham *et al.*, 1987) or 'variable' (Sheridan and Flowers, 1990) motor system or a more fundamental feature of the disorder. These issues need to be assessed in future studies.

In addition to possible differences in subject strategy, the duration of the S1-S2 interval may have contributed to inconsistencies in published results. Even with the present RT tasks which combined high compatibility between precue, imperative stimulus and response, the patients' mean precued CRT (Fig. 1*c*) was equivalent to SRT (Fig. 1*a*) only at the longest S1-S2 interval of 3200 ms. The shortest S1-S2 interval (200 ms) was not sufficient even for the controls to use advance movement parameter information to reduce their CRTs to the level of SRT. Therefore, in previous studies where S1-S2 intervals of 250 ms to 2000 ms have been used (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Stelmach *et al.*, 1986; Pullman *et al.*, 1988, 1990), the shorter intervals may not have been sufficiently long to allow full use of advance movement parameter information. The use of a single movement parameter in the precued two choice RTs of some of these studies (Evarts *et al.*, 1981; Bloxham *et al.*, 1984; Pullman *et al.*, 1988, 1990) may have meant that S1-S2 intervals required for full processing of the precue information need not be as long as in the present study. Without explicit test of the assumptions, however, fully cued CRT cannot be *a priori* considered equivalent to SRT. With the present stimuli and responses, the results clearly show that with an S1-S2 interval of 200 ms, partially and fully cued four CRT are the same as uncued CRT both for the patients and controls.

In one respect, the long S1-S2 intervals needed to fully utilize the advance information was surprising. Taking the patients as an extreme case, it was only with the 3200 ms interval that there was evidence of full preprogramming of one of a set of four responses. However, when the same programming is required after the imperative stimulus, as in the uncued CRT condition (Fig. 1*b*), the patients required only an extra 147 ms relative to SRT. The same dissociation can be seen, albeit less dramatically, in the control group, who required an extra 87 ms. In both groups, these 'automatic' processing times were much faster than time required for the 'conscious' processing of advance movement parameter information. This finding also suggests that in previous studies, fully precued CRT which is based on 'conscious' use of advance information cannot be equated in its time course with 'true' SRT which involves release of a preprogrammed response with the onset of the imperative stimulus. A distinction between the two tasks is also implied by the finding that MT in SRT is consistently faster than MT in precued CRT in both patients and controls.

Given sufficient time, 3200 ms with the present stimuli and responses, patients with Parkinson's disease could preprogramme a response. However, even with such an S1-S2 interval, the RTs of the patients were still slower than that of controls. The same degree of slowing was also present in the 'true' SRT condition. In addition, the patients were slower than the controls in the uncued CRT (not involving the use of advance information). The most parsimonious explanation of the slowness of the patients with Parkinson's disease relative to the normal across these conditions is a slowness in a stage of processing common to all of them; namely, response initiation. What processes underlie this deficit in response initiation in Parkinson's disease? Conduction along corticomotor neuron pathways has been shown to be normal in Parkinson's disease (Dick *et al.*, 1984). The size of the first agonist burst is, however, reduced (Hallett and Khoshbin, 1980). On the basis of these findings, it can be suggested that abnormal force production may be at least partly responsible for the slowness of response initiation in Parkinson's disease. However, as muscle 'energizing' and 'timing' processes are separate functions and as the two types of deficit do not necessarily coincide (Hallett and Khoshbin, 1980), deficits in muscle 'timing' may also account for the slowness in response initiation.

Finally, the differentially greater slowness of the patients on the high compatibility uncued CRT condition of the present study requires explanation. It is possible that in addition to the deficit in SRT the patients may be impaired on a stage of processing that is unique to uncued CRT; that is, either stimulus identification, decoding of S-R mapping rules or response selection (Fig. 1*b*). The focus of past research has been solely on investigating motor programming as the stage of processing that may be impaired in Parkinson's disease. The present results suggest that patients with Parkinson's disease may have additional deficits in these other stages of processing. These conclusions relating to SRT, fully cued and uncued CRT need to be addressed in future studies.

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HUMAN FUNCTIONAL ANATOMY OF VISUALLY GUIDED FINGER MOVEMENTS

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SUMMARY

The functional anatomy of visually guided movement was investigated in 18 normal subjects performing visuomotor tracking tasks during positron emission tomography imaging. Tracking a moving target with the index finger defined a network of focal responses of relative cerebral blood flow (relCBF) located in the primary motor cortex, dorsal parietal cortex, precuneate cortex, supplementary motor area (SMA) and ipsilateral anterior cerebellum relative to visual tracking alone. When the temporal complexity of the tracking task was altered by introducing a 'no go' contingency that allowed for greater time for movement preparation, there was a significant increase of relCBF in the SMA ($P < 0.0001$). When the spatial complexity was augmented by adding a secondary target that provided directional cues for the primary target, there were additional significant increases of relCBF in bilateral dorsal parietal cortex ($P < 0.05$) and precuneate cortex ($P < 0.05$). Although the cued 'no go' task was subjectively easier, relCBF responses were similar to the uncued 'no go' task in motor areas. Performing the tracking task with different body parts produced somatotopically distributed responses in only the motor cortex. The findings are concordant with clinical reports of patients with brain lesions and physiological evidence that identifies this distributed network for performing visually guided movement. The results provide direct human evidence in the normal brain that the supplementary motor area contributes in part to the sequencing of movements and the medial and dorsal parietal cortex participates in the integration of spatial attributes during selection of movements.

INTRODUCTION

Accurate, visually guided movement is a critical motor skill that is necessary for survival in most primate species. The simple act of reaching into extrapersonal space to touch a moving target requires a complex series of integrative steps that decode a vast amount of retinal information and culiminate in the recruitment of appropriate motor neurons for directed limb movement (Jeannerod and Prablanc, 1983). A rapidly growing literature has elucidated the sensory and motor extremes of this process; that is, visual analysis and motor control in both humans and non-human primates (Brooks, 1986; DeYoe and Van Essen, 1988). Concurrent processing streams direct visual information from striate cortex into visual association areas along multiple interconnected pathways. In non-human primate species, the connectivity maps of these parallel processes may be reduced to two paths necessary for the conversion of simple visual cues into associated useful attributes (DeYoe and Van Essen, 1988). The first, or 'what' pathway, is principally involved with object recognition and relies on visual cues of hue, binocular disparity

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and orientation selectivity (DeYoe and Van Essen, 1988). In the macaque species, for example, the path courses from the primary visual cortex to unimodal association area V4 into the inferotemporal cortex (Zeki, 1980; Maunsell and Van Essen, 1983; Ungerleider and Desimone, 1986). An analogous pathway has been proposed in humans, based on observations from brain lesions, functional imaging studies with positron emission tomography (PET) and electrophysiological experiments (Pearlman *et al.*, 1979; Damasio, 1985; Lueck *et al.*, 1989; Corbetta *et al.*, 1990; Zeki *et al.*, 1991). In humans the putative 'what' pathway extends in a cascade of processing centres from V1 into inferior occipital areas such as the lingular gyrus and lateral occipital cortex and then into the middle temporal cortex (Corbetta *et al.*, 1990).

The second, or 'where' pathway is critical for localization of objects in extrapersonal space and dependent on visual cues of hue, binocular disparity, orientation selectivity and movement selectivity (DeYoe and Van Essen, 1988). In the macaque species, information in this stream passes from striate cortex to inferior parietal cortex via area V3 and middle temporal cortex (Maunsell and Van Essen, 1983; Ungerleider and Desimone, 1986; Logothetis and Schall, 1989). An analogous pathway has been proposed in humans and courses from V1 into the inferior parietal lobe (Miezin *et al.*, 1987, 1988; Corbetta *et al.*, 1990).

At the opposite end of this neural network for visually guided movement, motor plans must be generated, executed and compared with ongoing movements. It has been suggested that the supplementary motor cortex initiates movements and possibly functions as an 'executor' for the sequencing of motor plans (Orgogozo and Larsen, 1979). The concept of 'executor' is based in part on the temporal differences in firing of supplementary motor area (SMA) neurons that are active prior to motor neurons (Freund, 1984). Premotor cortex activity has also been examined in great detail. One function is to interpret sensory attributes that represent triggers for the performance of previously learned patterned motor plans (Halsband and Freund, 1990). Prefrontal cortex has a more complicated role in motor control. For example, motor plans requiring temporal sequencing of movement in a correct order in response to sensory stimuli require a functioning prefrontal cortex (Goldman-Rakic, 1987; Fuster, 1989). Other areas are clearly functioning in tandem with the primary motor cortex. For example, ongoing 'feed forward' and 'feedback' adjustments for variations in force and position that keep motor plans within executable bounds are likely computed in lateral and parasagittal cerebellar zones (Thach, 1978; Stein, 1986; Leiner *et al.*, 1987; Inhoff, 1989). The degree to which each of these areas acts 'upstream' rather than in parallel with the primary motor cortex has not been established

A major, long efferent association fibre track projects from dorsal parietal cortex into premotor cortex, prefrontal cortex and lateral cerebellum providing the necessary bridge between visual and motor association cortex (Fuster, 1989). Together, these cortical areas define a distributed set of processing areas required for the performance of visually guided movement. Somehow, the visual attributes in the first part of this network must be converted to a functional state necessary for action within the motor system. The goal of the current study was to investigate the functional anatomy of this network by using PET measures of relative cerebral blood flow (relCBF) as an estimator of local neuronal activity.

Given the multiplicity of pathways in this putative network, extending from visual cortex to motor neuron, we considered it unlikely that any single point in the brain would be responsible for integrating visual information into discrete motor plans. Rather, it is likely that this conversion occurred in both 'motor' and 'sensory' association areas, cerebellum and subcortical nuclei in a dynamic, parallel manner. From prior electrophysiological experiments in non-human primates, we were particularly interested in the role of the posterior parietal lobe in this conversion along the 'where' pathway (Mountcastle *et al.*, 1975; Yin and Mountcastle, 1977; Jeannerod and Prablanc, 1983). We hypothesized that the activity of neuronal populations in this network would vary as a function of higher order contingencies demanded of our subjects while they performed a set of visually guided motor tasks. We developed a set of stimulation paradigms with different temporal and spatial complexities for comparison. We also varied the body part used to complete the visuomotor task, to examine the homuncular representation of the motor system and also to serve as an internal control. Theoretically, upstream or 'executor' motor areas and sensory association areas might not differ in the magnitude of their responses when different body parts were used during a tracking task.

We structured our control studies to account for three factors that could potentially confound our interpretation of local relCBF responses during the performance of visually guided movement. The first factor was the activation of cerebral cortex associated with smooth pursuit and saccadic visual tracking that might occur independently of any intended limb movement (Yin and Mountcastle, 1977; Miezin *et al.*, 1987, 1988; Godoy *et al.*, 1990; Morrow and Sharpe, 1990). Since we were interested in the organization of directed limb movement, this factor was controlled for by having the subjects perform the same tracking task with eyes alone. The second factor was the effect of attention (Spitzer *et al.*, 1988). Evidence suggests that attention to extrapersonal space is executed as a dispersed, parallel distributed process with nodes located in the frontal eye fields, the inferior parietal lobe and the cingulate gyrus (Posner *et al.*, 1988; Mesulam, 1990). Previous PET studies have demonstrated that heteromodal and 'upstream' unimodal visual association cortex responses are modulated by attentional mechanisms (Corbetta *et al.*, 1990). We attempted to disassociate the relCBF responses associated with attention to extrapersonal space from responses secondary to the process of visuomotor planning, by instructing our subjects to perform an active control study. This consisted of visual tracking to a matched target rather than a more passive control state such as sensory deprivation. Finally, we used a target with a constant form, colour and intensity for both the stimulation and control studies to minimize potential activation along the 'what' pathway.

METHODS

Imaging

Images of relCBF were obtained in 18 normal subjects while they performed a series of visually guided motor tracking tasks. Arterial sampling was not performed so that subjects would have at least one untethered arm to perform the motor tasks. Each subject received six intravenous bolus injections of 50 mCi of $H_2{}^{15}O$ commensurate with dynamic PET imaging with the Siemens/CTI 831-08 tomograph (Siemens Corporation, Hoffman Estates, IL, USA). The device collects 15 contiguous planes, 6.75 mm thick, with an in-plane resolution of 6 mm full width half maximum (FWHM). The final images were reconstructed with a low resolution filter (13 mm FWHM) for the calculation of percent change images and a medium resolution filter (9.5 mm FWHM) for anatomic localization (Figs 1, 2). Each emission image was corrected for attenuation using a transmission image of the subject collected in the same position with a Germanium-68 ring source. Head immobilization was achieved with a custom foam mold (Smithers Corporation, Akron, OH, USA).

Estimates of relCBF were based on a modified autoradiographic method (Herscovitch *et al.*, 1983; Raichle *et al.*, 1983). Five second frames were collected for 2 min beginning at the time of injection of $H_2{}^{15}O$. The first frame showing the arrival of cerebral radioactivity was identified. From this time point the counts for the ensuing 70 s were summed to generate images of relCBF. Difference images demonstrated an optimal signal to noise ratio at approximately this scan duration (Mintun *et al.*, 1989; Grafton *et al.*, 1990). Relative CBF images have a near linear relationship with absolute CBF and provide a reliable estimate of local CBF with a coefficient of variation of 2.54 for small sized (<2 cm^2) regions (Fox *et al.*, 1984; Mazziotta *et al.*, 1985*a*) The average number of counts from each global relCBF image were determined and each image was rescaled on a pixel by pixel basis to a common mean of 50 ml/100 ml/min. Effects of this normalization were assumed to cause relative underestimations for local increases in CBF and hence induce a conservative error. Since global changes of CBF are less than 5% when sensory deprivation is not used as a control state, the global effect on regional responses was assumed to be small (Cameron *et al.*, 1990).

Eight subjects underwent magnetic resonance imaging (MRI) scanning with an inversion recovery protocol (TE = 30 ms, TI = 300 ms and TR = 1275 ms) that generated 30 contiguous planes of data with an interslice distance of 4.4 mm and in-plane resolution of 1 mm (Fonar Systems, 0.35 Tesla, Melville, NY, USA). The MRI scans were characterized as normal by an experienced neuroradiologist.

Subjects

All subjects were studied after giving informed consent in accordance with the UCLA Human Subject Protection Committee. Eighteen normal subjects (17 male, 1 female; mean age 23 yrs) were studied. All subjects were free of medications for at least 6 wks, with no history of nervous system disease, and were normal on detailed neurologic examination. Handedness was determined with a standardized questionnaire and the dominant side was used for performance of tasks unless otherwise specified (15 right-handed, 3 left-handed subjects) (Raczkowski and Kalat, 1974).

Tasks

Three different stimulation paradigms were presented to subjects from two distinct groups (Group A, n = 12; Group B, n = 6). Table 1 summarizes the organization of the tasks and comparisons of the groups For the first stimulation paradigm, which we term 'continuous tracking', the subjects were presented with an identical randomly moving 0.5 cm target displayed on a 13 in colour video monitor coupled to a Macintosh IIcx computer (Apple computers, Cupertino, CA, USA). The visual fields peripheral to the computer screen were shielded to eliminate potential visual distractions. The target, moving in a fixed window of 6×8 cm, had a velocity of 20.6 cm/s and changed direction randomly (an average of 200 times per min). There was an imperceptible pause of 80 ms for computer calculations prior to target displacement in a new direction. The scanning was performed in a dimly lit room where the only ambient sound was from a tomograph fan.

Twelve Group A subjects tracked the continuous tracking target with their dominant index finger. The subjects held an arm outstretched, with the index finger lightly touching the screen They were instructed

TABLE 1 SUMMARY OF STIMULI AND VISUOMOTOR TASKS

	Visual stimulus	*Comparison*	*Number of subjects*
Group A	Continuously moving target (CT)	Index finger tracking—CT control	12
		Tongue tracking—CT control	5
		Toe tracking—CT control	4
		Non-dominant index finger tracking—CT control	2
Group B	Intermittently moving target (IT)	Index finger tracking—IT control	6
	Intermittently moving target with directional cues (ITC)	Index finger tracking with directional cues—IT control	6

Control = active tracking of the visual stimulus with only eye movements.

to keep the moving target covered as much as possible. The axis of rotation was about the first metacarpophalangeal joint. The hand was lightly braced against the video terminal for stability and the subjects were allowed to practise the tracking until only the first finger of their dominant hand slid gently over the target without any jumping movements.

Subjects practised the motor tasks for approximately 5 min prior to PET imaging. In a previously reported reproducibility study of continuous tracking with the dominant index finger, we found no evidence of a systematic change of the peak relCBF response with repeat measurement to suggest an effect of practice or habituation (Grafton *et al.*, 1991). Each task was then performed for 2 min beginning with the bolus injection of 50 mCi of $H_2{}^{15}O$ and dynamic imaging. The subjects were monitored by visual inspection for any axial or appendicular movements other than the specified body part. They were also rated for their performance in following a target and rewarded monetarily if the target was covered greater than 50% of the time.

Two of the Group A subjects tracked the target twice with the dominant hand and twice with the non-dominant hand.

Four of the Group A subjects followed the continuous tracking target with the great toe ipsilateral to their dominant hand. The leg was completely relaxed at the hip and knee and lay in extension. Flexion and extension of the great toe and rotatory movements at the ankle allowed for accurate tracking. Because the computer screen was closer to the subject's eyes than to the toe, the subjects did not have visual feedback from projection cues to confirm accurate spatial displacement of the toe in reference to the moving target on the computer screen.

Five of the Group A subjects tracked the continuous tracking target with their extended tongue. They were coached not to use their lips, face or limbs in concert with the tongue movements.

Each of these 12 subjects also performed matching control studies, during which the subjects were instructed to follow the movement of the same target with their eyes only.

The six subjects from Group B performed only index finger tracking, as opposed to other body parts, in response to two other stimulation paradigms (Table 1). In the first of these two paradigms, termed 'intermittent tracking', the same target as in the continuous tracking task was presented, and moving at the same speed of 20.6 m/s. The target changed direction less frequently, at 75 times per minute. Each target displacement ended with a 580 ms pause of fixed duration prior to the next displacement in a new direction. The subjects were instructed to keep the target covered with their dominant index finger. The task required finger tracking alternating with stationary target fixation and anticipation, and thus defined a 'no go' contingency.

In the other Group B stimulation paradigm, termed 'intermittent tracking with directional cue', the same primary target, 'no go' contingency and instructions were presented. In addition, a smaller (0.25 cm diameter) secondary target was presented. The secondary target would appear at the next intended site of displacement of the primary target 500 ms prior to the initiation of movement of the first target. Thus, the secondary target provided direction and distance cues for the accurate tracking of the primary target. Subjects were instructed not to jump ahead to the secondary target, but rather to keep the primary target covered at all times. The secondary target was also present in the intermittent tracking task to balance the number of targets presented between the two states; however, in the intermittent tracking task it lagged behind the primary target (500 ms) and provided no directional information.

Each Group B subject performed the intermittent tracking and intermittent tracking with directional cue tasks twice. Control images of eye movements alone were obtained for both the intermittent tracking and the intermittent tracking with directional cue tasks.

Image analysis

Percentage change of relCBF between two globally normalized images, where percent change = [(Stimulus − Control)/Control] × 100, were calculated within each subject on a pixel by pixel basis. Only increases in CBF were analysed for this report. Each percentage change image was analysed for misregistration in two ways. The first was to calculate the coefficient of variation of the distribution of all pixels in the brain on the percentage change image. A coefficient of variation greater than 30% was found to indicate misregistration. Also, the percentage change images were inspected for rims of activity along the cortical surface or as a halo over the dorsal portion of the brain that would occur with misregistration. Images from three subjects who would have been in the group of 18 subjects demonstrated these findings and they were excluded from this report.

Image analysis was limited to brain regions hypothesized a priori, to be involved in the performance of visually guided movement. To define foci in these regions, peak sites of increased relCBF were directly identified on the percent change images of the continuous tracking and intermittent tracking with directional cue tasks in the bilateral primary motor cortex, dorsal parietal cortex, precuneate cortex, SMA and ipsilateral anterior superior cerebellum. All of the motor and cerebellar foci were statistically significant when tested with a Z-score analysis for detection of outliers in a difference image between one continuous tracking task and two control states for each Group A subject (Fox *et al.*, 1988). Responses in parietal regions were near significance in Group A subjects and reached significance in the Group B subjects. The latter group had two studies per condition for comparison, and the associated reduction in variance increased the sensitivity to detect the parietal responses. Responses in premotor and postcentral gyrus cortex were not included because of the close adjacency to the primary motor cortex and partial volume effect. Although there may have been additional sites of significantly increased relCBF throughout the brain, the study design was blinded to them and they were not included in this analysis.

To verify the location of each focus noted above, the three-dimensional spatial coordinates for each were recorded and mapped onto the same subject's unsubtracted relCBF images (reconstructed with a high resolution filter of 9.5 mm FWHM for improved anatomical localization). The foci were also mapped onto MRI scans in the eight subjects with matching MRI sets (Pelizzari *et al.*, 1989). This localization approach avoided the use of stereotactic translations of images from different subjects onto a generic atlas, a procedure that could potentially propagate errors in the identification of specific gyral anatomy (Fox *et al.*, 1985*a*). To do the registration, contours at the skin surface obtained from the MRI and the PET transmission scan sets were aligned (Pelizzari *et al.*, 1989). The PET percentage change images were then resliced to match the MRI set to allow for accurate anatomical localization of intrasubject cortical responses. An unsubtracted $H_2{}^{15}O$ study from each subject was also resliced and compared with the MRI scan to verify the accuracy of the registration and alignment between the two study modalities.

The precentral gyrus and anterior superior cerebellum could be identified directly from the unsubtracted high resolution axial relCBF image or MRI (Fig. 1). To confirm localization of parasagittal foci, the unsubtracted relCBF image, MRI and percentage change images were resliced into 3 cm thick sagittal sections with the centre slice centred at the midline and, thus, including cortex from both hemispheres (Fig. 2). This was done using the 'Explorer' program and the Apple Macintosh computer (software developed

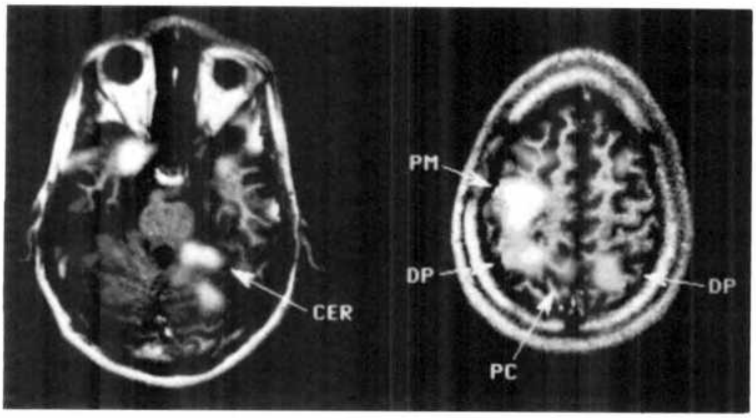

FIG. 1. Functional anatomy of continuous target tracking with the dominant (left) index finger. The PET relCBF images have been matched and registered with an MRI study from the same subject. All positive increases of relCBF (in white scale) are superimposed on axial MRI sections. A large focus is present in the right hemisphere spanning the precentral gyrus, the premotor area (PM) and extending into the postcentral gyrus. The peak site of response for this focus was in the precentral gyrus. Foci of activation are also present in the precuneate cortex (PC), and bilateral dorsal parietal (DP) cortex. Subtle increases of CBF are also visible in bilateral dorsolateral prefrontal cortex; however, this was not a consistant finding across subjects and was not included in the analysis. The inferior section demonstrates a response in the ipsilateral anterior cerbellum (CER).

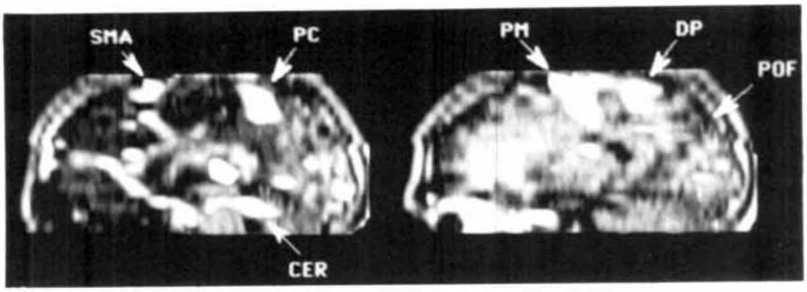

FIG. 2. Sagittal sections of relCBF and superimposed positive increases of CBF (in white scale) in a subject peforming the continuous tracking task with the dominant index finger. Images have been resliced in a standardized manner to 3 cm thick sagittal planes. The midline section (*left*) demonstrates SMA and precuneate cortex (PC) foci anterior and posterior to the central sulcus, respectively, and an anterior cerebellar (CER) response inferior to the tentorium. The parasagittal section (*right*), passing through the dorsal parietal cortex, demonstrates dorsal parietal (DP) and superior motor cortex premotor area (PM) responses. The parieto-occipital fissure (POF) is visible as an additional anatomic reference. Foci are clearly detectable in other areas; however, they were not evaluated in this study.

by Osman Ratib, Gerold Porenta, and Bill Kuhle, UCLA Division of Nuclear Medicine and Biophysics, Los Angeles, CA, USA) (Ratib and Huang, 1989). The pre- and post-central gyri, calcarine cortex, cingulate sulcus and parieto-occipital fissure were identified on the midline sagittal section of each unsubtracted relCBF or MRI study (Fig. 2). These structures formed boundaries for the regionalization of precuneate, cuneate and posterior boundary of the supplementary motor cortex. The average distance from the medial central sulcus to the SMA response was 2 ± 0.2 cm (Fig. 2).

We did not attempt to divide brain regions in parasagittal cortex into right and left sides because of the potential errors of quantification of closely placed foci on either side of the midline that would occur secondary to the partial volume effect in the low resolution percentage change images. Dorsal parietal cortex was defined on 3 cm thick sagittal sections located 3 cm lateral to the midline (Fig. 2).

After registration, alignment and reslicing of the PET studies, three-dimensional volume rendered images of the grey/white border of the MRI study were also created with Analyze program (Mayo Clinic, Rochester, MI, USA). The grey/white border was used for the MRI studies to allow for the visualization of deep sites of focal activation within sulci. Colour PET studies were then added at varying thresholds above a given percentage change in relCBF.

Task comparisons

Six of the subjects in Group A performed the index finger continuous tracking task twice and all of the Group B subjects performed the intermittent tracking and intermittent tracking with directional cue tasks twice. These paired studies were averaged together prior to calculation of percentage change images.

Table 1 summarizes the organization of the study comparisons. A direct comparison of Groups A and B motor tasks could not be made since they were from different populations. Therefore, each task was analysed with respect to a matching control. The relCBF change in each site of the visuomotor network defined above could then be compared across groups and conditions. This strategy assumed that the control studies uniformly corrected for effects of visual tracking, attention and non-motor factors across groups and conditions. For each Group A subject, the control study was subtracted from the matching continuous tracking motor task and percentage change images were derived. Although the intermittent tracking without and with directional cue tasks were both performed by Group B, a direct comparison of these tasks was not made. Instead, relCBF changes with respect to the intermittent tracking control image were calculated to facilitate comparison with the Group A results.

Response quantification

Estimation of the magnitude of a relCBF response is confounded by the partial volume effect in reconstructed PET images. Response size and magnitude cannot be simultaneously determined. If structure size can be estimated from anatomic imaging such as MRI then a better estimate of the true magnitude of a response can be obtained. This strategy was used for estimating the magnitude of responses in subcortical structures, as described below. For measurements of cortical responses, where the boundary of structures is less certain on anatomical images, this strategy is still in development. Therefore, we used the maximum percentage change of relCBF in each cortical focus to quantify local responses. This measure, although given as a change in magnitude, also reflects, to an unknown degree, a change in response volume. In a previous study we determined that the coefficient of variation for this measure is approximately 9% in subjects performing the same task serially (Grafton *et al.*, 1991). The variation in the site of the maximum response in the primary motor cortex, anterior cerebellum and SMA was also measured. Confidence intervals (95%) defining spheres of radii 5.4 mm, 4.4 mm and 4.2 mm were defined for these three sites, respectively. That is, any two foci could be considered as significantly distinct if separated by approximately 5 mm in any direction. However, the magnitude of two adjacent foci could not be quantified if they were closer than the instrument resolving power (FWHM = 12 mm).

Relative CBF responses in subcortical structures were measured with region of interest templates drawn on unsubtracted relCBF images or, when available, the matched and registered MRI scans over the right and left caudate, putamen and thalamus. Percentage changes in relCBF in these regions were then calculated. For brain structures spanning multiple image planes, the average relative increase in CBF, weighted by cross-sectional area in each plane, was calculated.

Statistical analysis

Between groups comparisons of the peak percentage change of relCBF responses were performed by analysis of variance (ANOVA). Inter-regional correlations were analysed by simple linear regression. A paired t test was used to compare the peak responses of relCBF, measured at the same loci, between the intermittent tracking and intermittent tracking with directional cue tasks.

RESULTS

Localization

Reproducible focal responses of relCBF could be identified in all subjects. For those subjects with matched and registered PET and MRI, the three-dimensional localization of PET relCBF responses were displayed directly onto MRI and rendered in three dimensions (Fig. 3). This imaging approach allowed for the rapid visual screening of cortical blood flow responses in single subjects and provided an overall impression of the distribution of cortical sites contributing to cerebral operations linked to the neurobehavioural tasks being performed. All of the tasks were associated with distinct relCBF responses in both ipsilateral and contralateral primary motor cortex, SMA, dorsal parietal, precuneate cortex and ipsilateral anterior cerebellum. The aggregate of these sites formed a functional network active during visuomotor tracking. The effect of changes in somatotopic, temporal and spatial parameters on each site of this network could then be examined.

Homunculus studies

Focal increases of relCBF in the primary motor cortex followed the classic sequential somatotopic representation as defined by Penfield (Penfield and Boldrey, 1937). Blood-flow responses secondary to toe movements were close to the interhemispheric fissure, those of finger movements were approximately two-thirds of the distance from the lateral fissure to the interhemispheric fissure and those of tongue movements were close to the lateral fissure.

Quantitative measures of the magnitude of focal responses in different cortical sites as a function of the body part used to perform the continuous tracking task are summarized

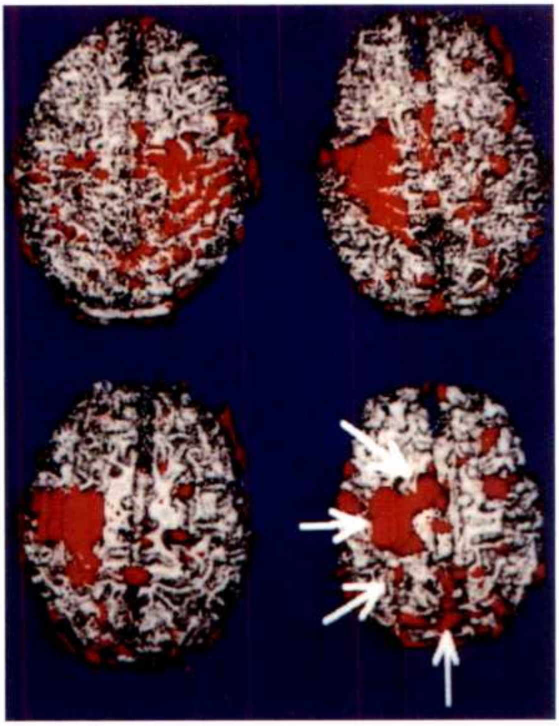

FIG. 3. The distribution of cortical responses associated with continuous finger tracking of the dominant index finger in three right-handed and one left-handed subjects (*upper left*). Three-dimensional surface rendered percentage change images of relCBF above a 10% increase (in red) have been mapped onto the same subject's registered and aligned MRI scan. The MRI images have been digitally edited so that the depicted surface is at the grey/white matter boundary. Frontal lobe is at the top and the subject's right hemisphere is to the right. The four arrows identify (clockwise, from bottom), the precuneate cortex, posterior parietal cortex, contralateral motor cortex/premotor area and contiguous dorsal parietal cortex, and SMA. This display technique allows for the rapid visual screening and interpretation of distributed blood flow responses associated with neurobehavioural tasks.

in Table 2. Although the subjects touched the screen with the index finger task and not the other tasks, there was no difference of the peak response in the contralateral primary motor cortex. Thus, the relative contribution of somatosensory input to the precentral cortex response was presumed to be minimal. Tongue movements were associated with symmetric motor cortex responses. Supplementary motor area responses did not vary as a function of body part; however, responses in the anterior cerebellum were significantly less during tongue movements. In the sensory association areas, there was no difference in the relCBF responses as a function of the body part used when the same continuous tracking stimulus was presented.

The hypothesis that discrete foci of increased relCBF were acting as a functionally connected unit could be examined by assessing the relative correlation of responses across subjects performing the index finger continuous tracking and eye tracking tasks. This definition of functional connectivity relies on a correlation of the magnitude of responses in spatially distinct sites, rather than a correlation of temporal responses. An analogous strategy has been used in several other recent PET investigations (Friston *et al.*, 1990;

TABLE 2. PERCENT INCREASE OF relCBF RESPONSES DURING PERFORMANCE OF CONTINUOUS VISUALLY GUIDED TRACKING WITH DIFFERENT BODY PARTS[1,2]

Cortical site	*Body part* *Index finger*	*Tongue*	*Toe*
Motor cortex			
Contralateral	35±9	34±7	40±13
Ipsilateral	17±8	35±9*	n.m.
SMA			
Bilateral	8±3	7±4	n.m.
Anterior-cerebellum			
Contralateral	n.m.		n.m.
Ipsilateral	32±9	19±8*	28±12
Dorsal parietal cortex			
Contralateral	15±5	11±5	10±4
Ipsilateral	11±4	8±2	9±5
Precuneate cortex			
Bilateral	10±3	9±2	12±6

[1] Values are percentage increase of relCBF ± standard deviation, measured at the peak site of response in each cortical site. [2] Between group comparisons by ANOVA, $*P < 0.05$. n.m. = not measured, *see* Methods.

Chollet *et al.*, 1991; Zeki *et al.*, 1991). These interrelationships were examined by linear regression (Fig. 4). Because we were correlating difference images, the analysis examined interrelationships across both brain states and subjects. Significant correlations were present between the contralateral motor cortex and (1) ipsilateral motor cortex ($r^2 = 0.4$, $P < 0.05$) and (2) bilateral SMA ($r^2 = 0.48$, $P < 0.05$). In contrast, ipsilateral cerebellar relCBF responses were close in magnitude to contralateral primary motor cortex relCBF responses and there was no significant correlation between the two foci ($r^2 = 0.1$, $P > 0.05$), suggesting either a functional independence between these two areas or insufficient methodologic fidelity (due to excessive variance) to demonstrate a correlation. Similar significant relationships were also present for the responses during the tongue continuous tracking task. Thus, the interrelationship of responses in the SMA and ipsilateral motor cotex with the contralateral motor cortex responses were independent of the body part used.

Response lateralization

To further examine the possibility of hemispheric lateralization and effects of hand dominance, two subjects performed the continuous tracking task twice with their dominant hand and twice with their non-dominant hand. When using their non-dominant hand, the magnitude of responses in the contralateral (right) and ipsilateral (left) motor cortex were similar to responses of the contralateral (left) and ipsilateral (right) cortex with the dominant hand. In contrast, both subjects demonstrated SMA and cerebellar responses that were greater than the expected 95% confidence interval when the same task was performed with the non-dominant compared with dominant hand. Parietal responses during tracking with the non-dominant hand were symmetric homologues to those with

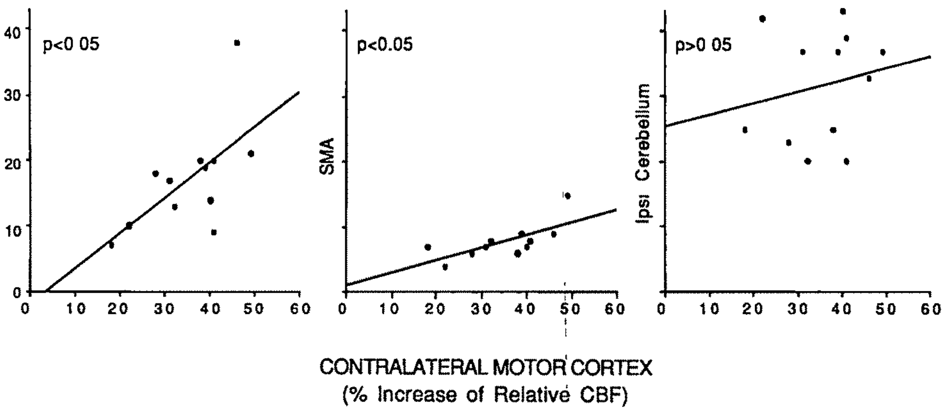

FIG. 4. Scatterplot of relCBF responses in the contralateral primary motor cortex versus responses in the ipsilateral primary motor cortex ($r^2 = 0.4$, $P < 0.05$), SMA ($r^2 = 0.48$, $P < 0.05$) and ipsilateral anterior cerebellum ($r^2 = 0.1$, $P > 0.05$). The data are from the 12 subjects performing the continuous tracking task with their dominant hand They demonstrate different degrees of covariance between different cortical motor areas, and suggest that the magnitude of response for some components of this network increase in parallel, as a functional unit

the dominant hand. That is, greater relCBF responses were present in the hemisphere contralateral to the limb performing the task.

Continuous versus intermittent tracking (dominant index finger)

The effect of altering the contingencies required to perform each of the motor tasks with the dominant index finger was analysed by performing a group comparison across the three stimulation paradigms with ANOVA. The results are summarized in Table 3. The major operational difference between the intermittent tracking task and

TABLE 3 PERCENT INCREASE OF relCBF RESPONSES DURING PERFORMANCE OF VISUALLY GUIDED TRACKING WITH THE DOMINANT INDEX FINGER[1 2]

Cortical site	*Continuous tracking*	*Intermittent tracking*	*Intermittent tracking with directional cue*
Motor cortex			
Contralateral	35±9	38±10	38±13
Ipsilateral	17±8	21±9	22±12
SMA			
Bilateral	8±3	13±3**	14±2**
Anterior cerebellum			
Contralateral	n m.	n.m.	n.m
Ipsilateral	32±9	31±7	30±7
Dorsal parietal cortex			
Contalateral	15±5	21±6*	24±8*
Ipsilateral	11±4	14±12	18±12
Precuneate cortex			
Bilateral	10±3	8±2	13±3*

[1]Values are percentage increase of relCBF±standard deviation, measured at the peak site of response in each cortical site. [2]Between group comparisons by ANOVA, *$P < 0.05$; **$P < 0.0001$. n.m. = not measured, *see* Methods

the continuous tracking task was the introduction of a 'no go' contingency. The addition of this temporally predictable attribute was associated with a significant increase of relCBF in the SMA ($F_{2,23} = 14.0$, $P < 0.0001$). Despite the decrease in the number of directional changes per minute with the introduction of a 'no go' contingency compared with continuous tracking, there were no differences in the magnitude of relCBF responses in contralateral motor cortex ($F_{2,23} = 0.14$, $P > 0.05$), ipsilateral motor cortex ($F_{2,23} = 0.67$, $P > 0.05$) or cerebellum ($F_{2,23} = 0.17$, $P > 0.05$). No significant differences were noted between the continuous tracking and intermittent tracking tasks in either dorsal parietal or precuneate foci. Therefore, the addition of a 'no go' contingency was reflected most dramatically by an increased response of the SMA.

Intermittent tracking with directional cues

Performance of the intermittent tracking task with directional cues added both vectoral and distance information; that is, visuospatial attributes, to the 'no go' intermittent tracking paradigm. With this task there were no significant between group differences of relCBF in any of the motor areas examined (Table 3). In contrast, there were significant increases of relCBF in contralateral dorsal parietal cortex ($F_{2,23} = 4.77$, $P < 0.02$) relative to the continuous tracking task, and in the precuneate cortex ($F_{2,23} = 3.66$, $P < 0.05$) relative to both the continuous tracking and intermittent tracking tasks.

Because the intermittent tracking and intermittent tracking with directional cue tasks were performed by a single group of subjects, a pair-wise comparison could be made to detect differences in foci related to these two tasks. The increased demand of visuospatial processing during performance of the intermittent tracking with directional cue task was associated with significant increases of relCBF in the contralateral dorsal parietal cortex ($P < 0.02$), ipsilateral dorsal parietal cortex ($P < 0.05$) and precuneate cortex ($P < 0.05$) relative to the intermittent tracking task (Fig. 5). The parietal response was greater on the contralateral (left) side in all six subjects.

After completion of the study, the subjects reported that the intermittent tracking task was subjectively easier to complete when the directional cue was provided. Despite this reported decrease in task demand on the subjects, the responses in motor cortex, SMA and cerebellum were similar in the two tasks (Fig. 6). Thus, the predominant effect of altering the complexity of the interpretation of visuospatial information was an increase of relCBF in bilateral dorsal parietal and precuneate cortex.

Subcortical responses

Quantitative region-of-interest analysis of the right and left putamen, caudate, globus pallidus and thalamus revealed no significant between group differences of relCBF associated with any of the tracking tasks. Regional responses ranged from 0% to 2% of increased relCBF, values that were not significantly greater than measures of image noise derived from control minus control comparisons. In addition, no asymmetries of relCBF were noted in subcortical structures. Correlation analysis demonstrated no significant linear regression fits between any subcortical and cortical structures.

DISCUSSION

Using a set of related tracking tasks, we examined a cerebral network that is active during the performance of a set of visually guided movements relative to visual tracking

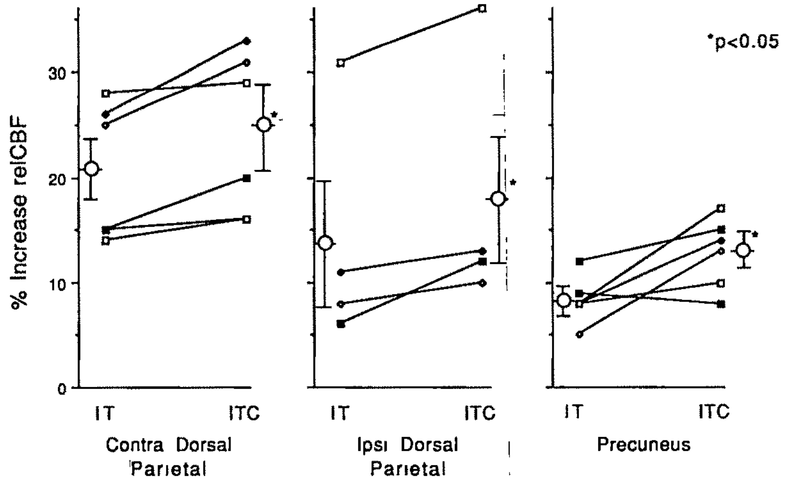

Fig 5 Scatterplot of focal relCBF responses in contralateral dorsal parietal cortex, ipsilateral dorsal parietal cortex and precuneate cortex for the six subjects performing the intermittent tracking (IT) and intermittent tracking with directional cue (ITC) tasks Foci in all three areas demonstrated a significant increase of relCBF by paired two-tailed t test when the visuospatial complexity of the task was augmented by the presentation of additional directional and distance information useful for goal-directed index finger tracking.

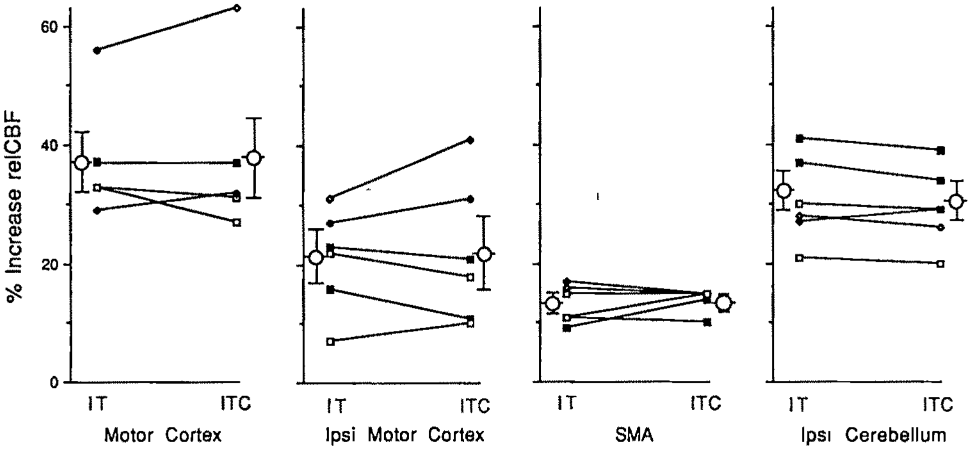

Fig 6. Scatterplot of focal relCBF responses in contralateral motor cortex, ipsilateral motor cortex, SMA and ipsilateral anterior cerebellum for the six subjects performing the intermittent tracking (IT) and intermittent tracking with directional cue (ITC) tasks Foci in all four areas demonstrated no significant change of relCBF by paired two-tailed t test even though the tasks were subjectively easier with the addition of direction and distance cues in the ITC task.

alone. The network is widely distributed, involving sensory association cortex, cerebellum and multiple motor areas. The common functional attributes measured across conditions that induce responses in this network include movement selection, sequencing and execution. Common functional attributes controlled for across conditions would include visual tracking, attention and non-motoric effects of the scanning environment.

We investigated the functional properties of each site of the cortical network by altering the somatotopic, temporal or spatial complexity in the neurobehavioural tasks. Our neurobehavioural paradigm design could not separate purely motor from sensory components to identify a discrete site of the brain where visual information was transformed into signals for motor control. That is, all four conditions measured effects of visual integration, motor selection and sequencing to different degrees. The findings show that different cortical domains are more or less active with various aspects of this visuomotor interaction. Since our analysis was performed on a limited set of brain areas, we cannot exclude the possibility that additional cortical sites are also involved in visuomotor processing. The properties of each of the brain regions that were investigated will be discussed in light of previously reported functional attributes suggested by the deficits associated with acquired lesions and neuronal responses determined by neurophysiological experiments and PET studies of these same areas.

Parietal lobe

The most interesting finding in this study was the enhanced activation of bilateral superior parietal and precuneate cortex during cued visually guided movement. Parietal responses were present for all the visuomotor tasks, irrespective of the limb used or whether there was a somatosensory feedback or not. They were significantly greater during intermittent tracking than continuous tracking. Of note, parietal responses were maximal when a directional cue was also introduced. This task and matching control were designed so that the subject would have to select and integrate useful visual information into a pre-existing, goal directed motor plan. Previous PET studies have demonstrated activation of the dorsal parietal region during visually directed movement. In early studies, effects of attention, eye movements, visual scanning and motor performance were not controlled for and thus interpretation of the specific contribution of dorsal parietal cortex to visuomotor processing could not be deduced (Roland, 1984). A recent PET study of the selection of movement in which these potentially confounding variables were carefully controlled for, has also demonstrated robust responses of CBF in the superior parietal cortex (Deiber *et al.*, 1991). Responses in this area were present whether the task was driven by internal or external (auditory) cues compared with fixed movements, suggesting a critical role of this area for movement selection, rather than movement execution. Our results using visual cues demonstrate that this site, along with the precuneate cortex, is critical for movement selection based on the integration of visual information as a step in generating an executable visually guided motor plan. The combined PET results suggest that the responses in bilateral dorsal parietal cortices during selection of movement are in part modality-independent, since they occur with both visual and auditory external cues as well as internal cues. Further studies may define whether there are modality specific subregions within the superior parietal cortex.

Localization of PET relCBF responses to bilateral dorsal parietal and precuneate cortex during visually guided movement are concordant with the clinical state known as 'optic ataxia'. This sign, manifest as an inability to point or reach accurately at a target under visual guidance, was first decribed by Balint in a patient with bilateral occipital-parietal lobe lesions (Balint, 1909). The patient also had defects of impaired visual scanning (ocular apraxia) and visual disorientation (simultagnosia) and represents the hallmark case for Balint's syndrome (Balint, 1909). Since that report, it has been repeatedly demonstrated that optic ataxia can occur in isolation with either unilateral or bilateral posterior parietal lesions (Garcin *et al.*, 1967; Rondot and de Recondo, 1974; Boller *et al.*, 1975; Tzavaras and Masure, 1976; Rondot *et al.*, 1977; Levine *et al.*, 1978; Damasio and Benton, 1979; Auerbach and Alexander, 1981; Denes *et al.*, 1982; Ferro *et al.*, 1983; Ferro, 1984; Perenin and Vighetto, 1988). The findings of optic ataxia are distinct. It is a visuomotor disorder without visual field loss, inattention, neglect or visual space misperceptions. It is modality specific; patients preserve accuracy in pointing to sounds or to parts of the self by using audio or somatosensory cues for spatial orientation. Elementary motor function is unaltered; however, in a fraction of cases there may be hypokinesia, slight weakness, abnormal resting arm posture or slowing of alternating finger movements. The visuomotor deficit is seen in both distal and proximal limb components, manifest as errors in reaching, hand orientation and individual finger movements.

In the most extensive study to date, Perinen and Vighetto compared the location of abnormalities in eight patients with optic ataxia (Perenin and Vighetto, 1988). The lesion's most common location was in the contralateral interparietal sulcus extending into the superior parietal lobule and the precuneus (Fig. 7). The location is in excellent agreement with the site of superior parietal responses detected in our PET visually guided finger-

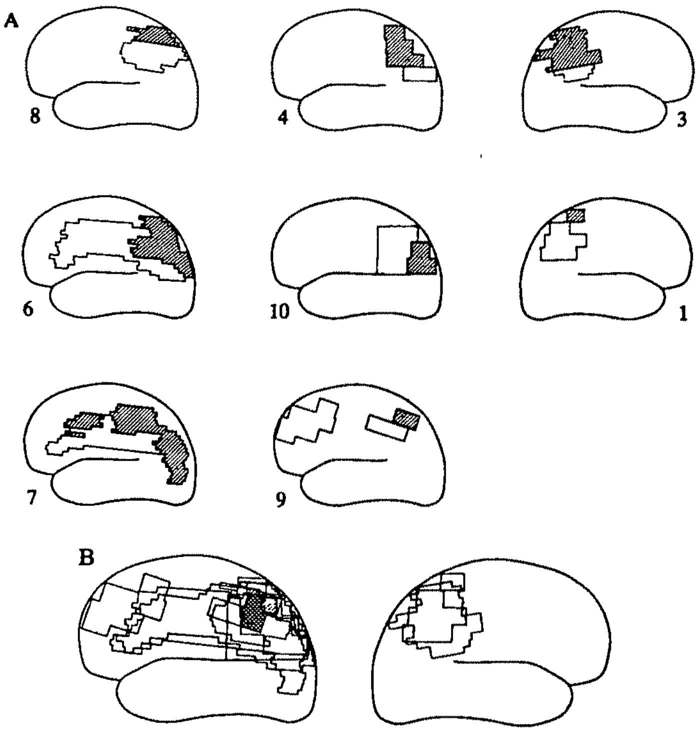

FIG. 7. Reconstruction of the CT location of parietal lesions in eight patients with optic ataxia. The common locus is in the region of the interparietal sulcus, superior parietal lobule and precuneus, similar to the responses obtained with PET during visually guided movement (adapted from Perenin and Vighetto, 1988). A, individuals; B, group composite

tracking tasks, demonstrating a close correlation between PET functional responses and clinical deficits from lesion studies.

Functional comparisons of our parietal lobe PET relCBF responses with electrophysiological studies of single unit recordings in non-human primate species should be made with some caution. The PET relCBF measures of cerebral 'activation' measure large neuronal populations with functional properties that are more general than those derived from single neuron studies. Ideally the attributes of single neurons should be concordant with responses associated with PET studies using similar neurobehavioural paradigms. Differences in the cytoarchitectonic organization of the dorsal parietal cortex between species must also be accounted for. For example, the human parietal lobe has considerably greater mass in Brodmann's areas 5 and 7 as well as additional gyri not present in other primate species, such as the angular and supramarginal gyri. Despite these complexities, important similarities exist between species.

Parietal lobe neurons of the macaque species have been parcelled into two domains corresponding to the superior (Brodmann's area 5) and inferior (Brodmann's area 7) parietal lobules. Neurons in the superior parietal lobule are active with passive joint rotation, deep tissue sensation and hand manipulation, amongst others (Mountcastle *et al.*, 1975). In humans, the dorsal parietal cortex adjacent to the postcentral gyrus (Brodmann's area 5) is active during somatosensory association tasks. Lesions at this site typically cause astereognosis and agraphaesthesia (Ginsberg *et al.*, 1987). This site is anterior to the superior parietal responses associated with our PET study, suggesting a functional disassociation of visual and somatosensory processing in the dorsal parietal lobe.

Unlike Brodmann's area 5, neurons of the inferior parietal cortex, in non-human primates, coresponding to Brodmann's area 7, are active during visual fixation, projection and hand manipulation, visual tracking, and recognition of visual space, amongst others (Mountcastle *et al.*, 1975). Lesions of this area that extend posteriorly to the parieto-occipital junction cause defects in exploration of extrapersonal space characterized by visual inattention and neglect, with or without visual field defects (Haaxma and Kuypers, 1974).

Visuomotor experiments of the superior and inferior parietal cortex (Brodmann's area 7) in humans compared with other primate species are best interpreted in the context of the functional anatomy of visual attention and goal-directed behaviour. Mesulam has recently reviewed the important role of the inferior parietal lobe (von Economo's area PG) as a node for directing attention to multimodal information travelling in parallel distributed processes (von Economo, 1929; Mesulam, 1990). The well-known deficits of hemineglect, simultagnosia and impaired visual scanning are all associated with lesions in this area, particularly when located in the right hemisphere. The PET studies in humans have also demonstrated activation of the inferior parietal lobe during attention to object velocity (Corbetta *et al.*, 1990). Our results support the hypothesis that the human posterior parietal lobe (Brodmann's area 7) may be partitioned into inferior and superior functional units corresponding approximately to the inferior parietal lobule and superior parietal and precuneate cortex. The former area is involved in directed visual attention and is an early 'node' (in the temporal sense) of the 'where' pathway for visual processing, critical for the recognition of movement and velocity. The superior parietal and precuneate cortex are necessary for integrating visual cues into movement selection, independent of the body part used to perform the task. Whether an analogous functional distinction is also present in other primates remains to be determined.

Additional evidence from lesion studies would suggest a dichotomous flow of visual information from inferior parietal and parieto-occipital cortex to the superior parietal lobe. Although focal lesions can cause optic ataxia without defects in visual scanning, the converse is not true. When other defects such as Gerstmann's syndrome, ideomotor apraxia, optic apraxia or neglect were also present with optic ataxia, the lesion typically extended to the inferior parietal lobe or parieto-occipital junction (Heilman *et al.*, 1986; Pierrot-Deseilligny *et al.*, 1986; Perenin and Vighetto, 1988; Trillet *et al.*, 1989). The findings suggest a dissociation of function necessary for the computation of visual information for the performance of visuomotor tasks. Visual attention must first be directed to an object before the object can be interpreted as a target that has value in the context of goal-directed behaviour (Jeannerod and Prablanc, 1983). Once this interpretation is made, a goal-oriented motor plan is generated. Both steps are likely gated by internal drives arising from limbic, cingulate and frontal cortical domains (Mesulam, 1981; Posner *et al.*, 1988).

Deficits associated with inferior parietal lesions in humans typically demonstrate functional lateralization with neglect more commonly associated with right hemispheric lesions (Heilman and Van Den Abell, 1980; Mesulam, 1981). We could not detect a similar pattern of hemispheric lateralization in the relCBF responses of the superior parietal cortex. Although the number of subjects performing the continuous tracking task with the dominant and then non-dominant hand was small, we found that posterior parietal responses were always greater in the contralateral hemisphere for both the dominant and non-dominant hand. The findings suggest a hand effect without hemispheric lateralization.

Since we used full visual field stimulation, field effects could not be examined directly. Most patients with optic ataxia have a unilateral injury to the dorsal parietal lobe and are impaired in reaching with either hand for objects located in the visual field contralateral to the parietal lesion, consistent with a visual field organization of this region (Perenin and Vighetto, 1988). In some, however, there is impairment of a single hand in one hemifield, suggesting a hand effect (Tzavaras and Masure, 1976; Auerbach and Alexander, 1981). We would propose that bilateral dorsoparietal cortex is comprised of a mixture of both hand and field sensitive neurons. This would be the cortical level at which extrapersonal hemispheric spatial information is transformed into an intrapersonal somatotopic representation. Long association fibres project from the dorsal parietal cortex into premotor and prefrontal cortex, providing the critical link from sensory to motor association areas from which motor action can be orchestrated.

Supplementary motor area

A role for the SMA in the sequencing of movement has long been postulated, based on observations from lesion studies, electrophysiological recordings, blood flow imaging and direct cortical stimulation (Ingvar and Philipson, 1977; Lassen *et al.*, 1977; Lauritzen *et al.*, 1981; Freund, 1984). Lesions within this dorsomedial site are associated with a severe reduction in spontaneous motor activity, arrest of propositional speech, impaired bimanual coordination, the development of mirror writing or movements and impairment of memory guided saccadic eye movements (Brinkman, 1981; Laplane *et al.*, 1981;

Goldberg, 1985; Chan and Ross, 1988; Gaymard *et al.*, 1990). The concept that the SMA operates as a 'supramotor' centre for the sequencing and initiation of movement is supported by the loss of spontaneous movement and speech after bilateral SMA injury (without paralysis), the presence of early negative potentials on scalp recordings emanating from this area (Bereitschaftspotential) and CBF imaging studies that have shown an increase of flow during both motor ideation without movement and with the performance of complex finger movements (Ingvar and Philipson, 1977; Orgogozo and Larsen, 1979; Roland *et al.*, 1980; Deecke, 1987). Our results are concordant with the concept that the SMA acts as a movement sequencer, independent of body part used to perform the task. We found a significant increase of relCBF in this region when the tracking task changed from a continuous to an intermittent 'no go' paradigm. In the latter case the subject is allotted greater time for movement preparation. Another recent PET result has demonstrated an analogous finding (Deiber *et al.*, 1991). A greater response was noted in the SMA when subjects were allowed to prepare a movement before an internally driven cue rather than an external cue. The two studies used different types of external stimuli, suggesting this function of the SMA is not modality specific. Our data would suggest that the SMA may not be involved with the integration of visuomotor targets into a motor plan. When a directional and distance cue was added to the 'no go' task, we detected no further augmentation of relCBF in this area. The SMA may be a sequencer of motor plans that are molded into more precise movements by other cortical areas utilizing visual, somatosensory, spinocerebellar and vestibular feedback.

Supplementary motor area responses may also reflect the difficulty of a motor task. We found a significant correlation between SMA and motor cortex responses across all subjects, suggesting they respond as a functionally connected unit. In addition, we found that SMA responses were significantly greater when subjects performed the same tracking task with the non-dominant hand, even though motor cortex responses were similar to the dominant hand. The non-dominant motor cortex may require a greater modulation by the SMA to perform an equivalent task than the dominant cortex. Characterization of regional interrelationships such as these will be critical for the interpretation of findings in patients with functional recovery after cerebral injury or developmental plasticity (Brion *et al.*, 1989; Calford and Tweedale, 1990; Chollet *et al.*, 1991).

Cerebellum

Previous mapping studies with PET have demonstrated increased CBF in the human cerebellum during patterned finger movements and with maintenance of hand posture (Fox *et al.*, 1985*b*; Colebatch *et al.*, 1990, 1991). When task execution is controlled for, responses are no longer significant (Deiber *et al.*, 1991). In our study, no differences were noted as the spatial or temporal complexity was altered. The common denominator of the PET anterior cerebellar responses, therefore, has been execution of motor tasks driven by either visual, auditory or internally programmed cues. The response in our study varied as a function of the body part used, with a diminished response during tongue (axial) compared with toe or finger (appendicular) movements, suggesting functional specialization for limb rather than axial movements. It has been proposed that this cortical region of the cerebellum serves as a 'comparator' concerned with the detailed execution of movement (Thach, 1968, 1978; Lundberg, 1971; Strick, 1983;

Stein, 1986; Leiner *et al.*, 1987; Lisberger, 1988; Inhoff *et al.*, 1989). The major afferents in this area are from the prefrontal and premotor cortex via cortico-ponto-cerebellar mossy fibres and the extrinsic spinocerebellar afferents providing proprioceptive information from the periphery (Vachananda, 1959; Tsukahara *et al.*, 1968). Desired limb position, commanded by motor cortical areas are compared with actual limb position, signalled by the proprioceptive afferents. Disruption of the outflow from this area by cooling of the interpositus nucleus causes a delayed and insufficient compensation in response to a mechanical torque pulse, leading to limb oscillation (Stein, 1986).

Precentral cortex

The magnitude of relCBF responses remained constant across somatotopic, temporal and spatial contingencies, suggesting a relative stability in the execution of the different motor plans. The robust response in the primary motor cortex allows for non-invasive mapping of the primary motor cortex in individual subjects. This may be applied clinically to patients with a number of cerebral disorders including arteriovenous malformations, neoplasms and epilepsy. The combination of PET and MRI can be used as a preoperative localization technique for optimizing resection boundaries.

Subcortical sites

No significant increases in regional relCBF were demonstrated in basal ganglia nuclei for any of the tasks. The absence of these basal ganglia responses in our study may be a function of the random visuomotor task we used. Our task was unpatterned and used an active control state with eye movements. Previous authors have demonstrated striatal and thalamic responses in subjects performing a patterned task requiring digit tapping in a complex sequence compared with a control state of sensory deprivation (Roland *et al.*, 1982; Mazziotta *et al.*, 1985*b*). Subcortical responses have also been noted in patients after recovery from stroke who perform similar patterned digit movements (Chollet *et al.*, 1991).

Methodological considerations

An implicit assumption of our report, among others, has been that a linear relationship between CBF and neuronal activity is present throughout heteromodal association cortex, motor areas and subcortical nuclei. It remains to be determined how cortical neurons control local CBF as a first step towards confirming this linear relationship throughout the neocortex. Comparative studies that combine electrophysiological and PET imaging methods in non-human primates or humans undergoing cortical mapping are needed to characterize the spatial and temporal features that couple flow and neuronal activity.

Our results demonstrate that functional attributes may be studied in a widely dispersed cortical network by minor alterations of a neurobehavioural paradigm. The PET approach allows for the simultaneous examination of a large-scale network, and provides a map that localizes the optimal sites for more detailed analysis with electrophysiological or biochemical techiques.

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ANATOMICAL ASYMMETRIES AND FUNCTIONAL LATERALITY

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SUMMARY

Anatomical and functional asymmetries were measured using MRI and dichotic, visual field and hand performance laterality tasks in 104 normal right- and left-handed male and female adults. Comprehensive linear and area measures showed consistently larger brains when handedness and language laterality were in the same direction. Right-handers with left cerebral dominance for dichotic listening and left-handers with right cerebral dominance had larger structures. Hand performance was the strongest functional laterality related to anatomical asymmetries. Left hemisphere dominance on this task was associated with larger right frontal and left occipital widths. Dichotic listening or visual field laterality alone was not associated with contralaterally larger temporal, parietal or occipital measurements. Left visual field dominance for reading, interacting with right-handedness, was associated with wider left parietal width.

The complex associations between anatomical and functional asymmetries contribute to individual differences in cerebral organization. The demonstrated association of coincident hand performance and language laterality measures with larger anatomical structures may confer biological advantage. Morphometric analysis *in vivo* may have increasing importance in interpreting behaviour.

INTRODUCTION

The complexity of functional lateralization is reflected by the large body of recent psychological literature dealing with dichotic listening, visual field preference and hand performance (for reviews *see* Bryden, 1982; Bradshaw, 1989; Hellige, 1990). Anatomical asymmetries have been reported sporadically since Heschl (1878) described two transverse temporal gyri on the right and only one on the left in the majority of human brains. The right hemisphere was usually reported to be larger and heavier (Heschl, 1878; Broca, 1885; Braune, 1891; Wilde, 1926; Hoadley, 1929; Gundara and Zivanovic, 1968; Schwartz *et al.*, 1985) although the opposite was found by others (Cunningham, 1892; Hrdlička, 1907; Hadziselimovic and Cus, 1966). Von Bonin (1962) was more impressed by the small magnitude of the differences and the relative symmetry of the two hemispheres. A larger left planum temporale was originally described by Pfeifer (1936) and subsequently confirmed and quantitated to be in 65% of the brains examined by Geschwind and Levitsky (1968). This finding is related to the Sylvian fissure asymmetry originally described by Eberstaller (1884) and Cunningham (1892), and more recently quantitated by Rubens *et al.* (1976).

Cytoarchitectonic asymmetries corresponding to anatomical ones were described on the planum temporale (Galaburda *et al.*, 1978) and in the primary auditory cortex

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(Seldon, 1985). Broca's area asymmetries were also reported using various methods (Wada *et al.*, 1975; Galaburda, 1980; Falzi *et al.*, 1982; Albanese *et al.*, 1989). A larger right frontal lobe was found in autopsy specimens (Weinberger *et al.*, 1982; Weis *et al.*, 1989). Subcortical structures were also observed to be asymmetrical with a larger left pallidum in most cases (Orthner and Seler, 1975; Kooistra and Heilman, 1988).

In vivo asymmetries in CT scans have been reported, namely that right-handers have greater left occipital and right frontal petalia (protuberance) and wider left occipital and right frontal lobes (LeMay and Kido, 1978). Left-handed subjects have either more symmetrical distribution or the reverse pattern. Chui and Damasio (1980), however, found no significant differences between right- and left-handers. McRae *et al.* (1968) found the left occipital horn of the lateral ventricle larger in right-handers but equal in left-handers. LeMay and Culebras (1972) found more equal parietal opercularization on the angiograms of left-handers. Hochberg and LeMay (1975) described larger left parietal operculum in 67% of right-handers and 21% of non-right-handers.

With the exception of a few studies providing empirical evidence, the anatomical basis of functional laterality remains hypothetical. Ratcliff *et al.* (1980) correlated the asymmetry of the Sylvian point on angiograms with intracarotid amytal studies of cerebral dominance in an epileptic population. A larger left parietal operculum was found in 66% of left positive WADA tests and 35% of right positive subjects. Strauss *et al.* (1985) found no correlation between Sylvian point measures and amytal testing of language dominance, but some correlation with right ear advantage (REA) on dichotic tests and posterior temporal widths. The Sylvian asymmetries were equally distributed. Witelson and Kigar (1988) reported pilot data of post-mortem cerebral asymmetry in cancer patients tested with dichotic listening before they died. Four patients with REA had larger left planum, while three with 'non-REA' had equal or smaller left planum.

Magnetic Resonance Imaging (MRI) provides an opportunity to observe anatomical asymmetries *in vivo* with an accuracy that approximates actual anatomical measurements. We demonstrated MRI asymmetries in the opercular region, with sharper demarcation on the right and more gradual transition from opercular cortex to parietal cortex on the left side in the majority of right-handers (Kertesz *et al.*, 1986). This pattern corresponds to the larger planum temporale on the left. Sagittal MRI cuts also revealed a longer left planum on MRI which is more so in right-handers (Habib, 1989; Steinmetz *et al.*, 1991). The right temporal lobe and hippocampus were larger in all right-handers on MRI (Jack *et al.*, 1989). Our study of both linear and area measurements on MRI documented the relationship of these anatomical measures to sex and handedness with right-handers having larger right anterior frontal and left parietal and occipital widths (Kertesz *et al.*, 1990). A sex by handedness interaction in the parietal area ratio measure showed larger left-sided measures in right-handed males and left-handed females.

The present study investigates the relationship between measures of functional laterality and anatomical measures of asymmetry. We postulated that functional measures of auditory language dominance would relate to temporoparietal and frontal asymmetries; hand performance to frontal and visuoverbal measures to parietal and occipital asymmetries.

METHODS

Subjects

Fifty-two right-handed (25 males, 27 females) and 52 left-handed (26 males, 26 females) university students and hospital employees, age 18–49 yrs ($\bar{x} = 26.92$, SD = 7.19) were included. Handedness was determined by self report on a handedness questionnaire (Bryden, 1982).

Imaging

All subjects had a set of inversion recovery and spin echo MRI scans in horizontal, coronal and sagittal orientations on a 0.15 Tesla Technicare resistive magnet. Axial cuts were aligned parallel to the Sylvian fissure with a laser light, using the orbitomeatal line as the external landmark, and subtracting 15°. Coronal cuts were perpendicular to the axial ones. To eliminate observer bias, the sides of the images were randomly reversed, and placed in an envelope, which masked identifying data. The images were projected by a photographic enlarger to life size to make linear measures, and to trace the areas selected.

The axial cut above the third ventricle was used to measure hemispheric sagittal length and area. The longer sagittal length was divided at 10% and 30% anteriorly and posteriorly, creating anterior frontal, posterior frontal, parietal and occipital widths (Fig. 1, left side of figure, labelled S). The first coronal cut, showing separate temporal lobes, was used to trace the frontal lobes (Fig. 2, left side of figure, labelled F). The temporal lobes were traced on the image where the pons and the medulla appeared to be continuous (Fig. 2). The inferior ramus of the Sylvian fissure was connected to the hippocampal sulcus by a straight line to outline the temporal lobe area (Fig. 2, labelled T). The parietal lobe was traced on the same image by connecting the superior ramus with the tip of the anterior horn of the lateral ventricle and the inferior end of the interhemispheric fissure (Fig. 2, labelled P). Brain contours were traced by bridging sulci when they were sharper than 90° and following them to their depths when they subtended a wider angle.

The areas were traced and digitized using a microcomputer with all measures done by two independent observers blind to both the functional data and the side of the scan. Because the correlations ranged from 0.88 to 0.97 between sets of measurements ($\bar{x} = 0.91$), the average of the two sets was used.

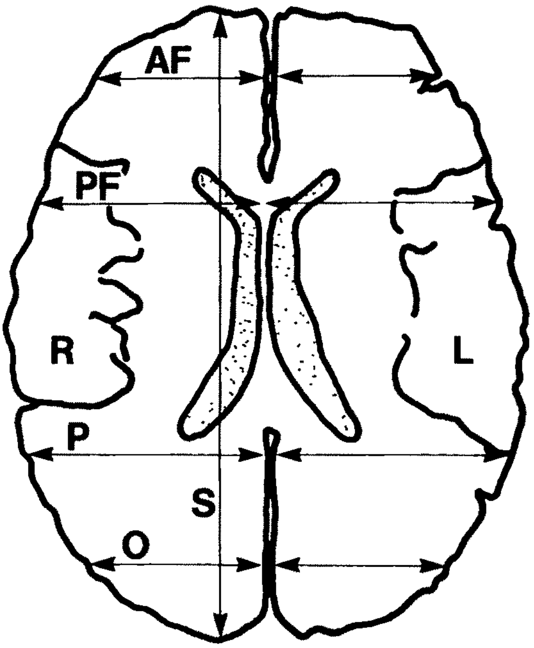

FIG. 1. Axial MRI image used for linear and hemispheric area measures. AF = anterior frontal width, PF = posterior frontal width, P = parietal width, O = occipital width, S = sagittal length, R = right operculum, L = left operculum. This is a tracing of an actual image, indicating asymmetry of posterior opercular angle (Kertesz *et al.*, 1986).

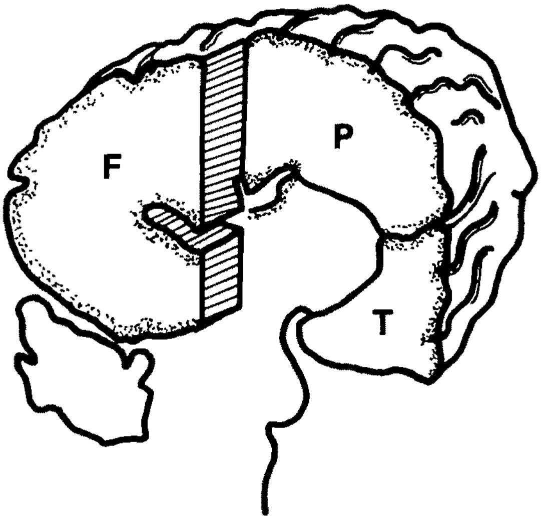

FIG. 2. Tracings of actual coronal MRI slices, illustrating the anterior-posterior location and the definition of the areas (*see* text for detail). F = frontal area, P = parietal area, T = temporal area.

Behavioural measures of cerebral dominance

Dichotic listening. Stop consonant-vowel pairs (e.g. Ba-Da) were presented over 30 practice trials and four blocks of 30 test trials. An attention-directed paradigm was used to measure laterality. The subjects were instructed to attend to items presented to one ear for 60 trials and to the other ear for the rest. A laterality index was derived from an odds ratio of the right ear responses exceeding left ear responses (Bryden and Sprott, 1981).

Visual half-field. A tachistoscopic presentation of a series of three- and six-letter, monosyllabic, high frequency words were presented unilaterally once in each visual field for 150 ms for a total of 24 practice and 80 test trials. A voice activated timer recorded reading latencies in milliseconds. This laterality index was derived from a slope formula subtracting the differences between the reaction times of six- and three-letter words for left visual field from six- and three-letter words for the right visual field (Bub and Lewine, 1988).

Hand performance. Subjects were instructed to mark a series of small circles with dots as quickly as possible in 20 s. This was performed twice with each hand. This laterality index was calculated using a ratio of the difference between number of dots completed by each hand divided by total number of dots (Tapley and Bryden, 1985).

Statistical analysis

Subjects were grouped by cerebral dominance for dichotic listening visual field and hand performance, as well as by gender and reported hand use. Frequency distribution statistics and multivariate statistics were used to examine group differences and the relationships of sex, handedness and laterality to the anatomical measures. Separate three-way factorial MANOVAs were performed on dependent sets of linear and area anatomical measures. Both uncorrected measures and asymmetry ratio measures, calculated using the formula (L−R/L+R), were used. Between subject factors were sex (male, female), handedness (right, left) and task laterality (left or right hemisphere dominance for a particular task). Selected main effects and interactions were tested: main effect of task laterality, task laterality by hand interaction, task laterality by sex interaction and task laterality by hand by sex interaction. Only significant main effects and interactions were reported for each laterality task.

RESULTS

Cerebral dominance in the laterality tasks

Cerebral laterality task results showed the majority of the subjects to be left hemisphere dominant on the presented tasks, as expected. On the dichotic task, 66%

of the males, 80% of the females, 71% of the right-handers and 74% of the left-handers were left hemisphere dominant. Sixty-nine per cent of the left-handers, 63% of the right-handers, 73% of the males and 60% of the females were left hemisphere dominant on the visual field reading task. None of these distributions differed significantly on a χ^2 analysis. Hand performance corresponded to self reported handedness without exception. The distribution is related to selecting equal numbers of left- and right-handed subjects (Table 1).

TABLE 1 CEREBRAL DOMINANCE FOR DICHOTIC LISTENING, VISUAL FIELD AND HAND PERFORMANCE

		Dominance	
Performance measure	*Subject group*	*Left*	*Right*
Dichotic listening	Males	33	17
	Females	39	10
	Rights	35	14
	Lefts	37	13
	Combined	72	27
Visual half-field reading task	Males	35	13
	Females	31	21
	Rights	31	18
	Lefts	35	16
	Combined	66	34
Dot task (hand performance)	Males	25	26
	Females	27	26
	Rights	52	0
	Lefts	0	52
	Combined	52	52

The distribution of linear asymmetries and cerebral dominance

The incidence of linear measure asymmetries was tabulated in each cerebral dominance group determined by dichotic listening and visual field preference separately by sex and by handedness (Table 2). All of the dichotically right dominant females had longer sagittal length on the right side, but only about two-thirds of the left dominant ones had the same asymmetry ($\chi^2 = 4.06$, $P = 0.04$). In other anatomical measures the distribution was more similar between the left and right dominant individuals. Males and left-handers more often had larger left anterior frontal widths regardless of cerebral dominance, while females and right-handers showed an even distribution. The posterior frontal widths were more often larger on the left side in the left dominant groups for dichotic listening. Regardless of cerebral dominance for the visual-verbal task, the males and left-handers were slightly larger on the left; females and right-handers were evenly distributed. Parietal and occipital width distributions showed no significant differences between left and right dominant individuals in dichotic or visual tasks, all showing a clear majority of R > L asymmetry (Table 2).

The distribution of area asymmetries and cerebral dominance

Hemispheric areas were generally larger on the right. This was more evident for males with left rather than right dominance for dichotic listening ($\chi^2 = 5.16$, $P = 0.02$)

TABLE 2 LINEAR ASYMMETRIES AND CEREBRAL DOMINANCE

Subject group	*Cerebral dominance*	*Sagittal length*			*Anterior frontal width*			*Posterior frontal width*			*Parietal width*			*Occipital width*		
		L > R	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*
On dichotic listening																
Males	Left	9	2	21	20	1	11	19	4	9	5	2	25	4	1	27
	Right	6	3	7	11	0	5	8	1	7	4	0	12	4	1	11
Females	Left	11	4	22*	16	4	17	20	3	14	5	2	30	6	1	30
	Right	0	0	9	3	3	3	4	1	4	3	0	6	1	0	8
Rights	Left	7	5	21	15	3	15	18	2	13	6	1	26	7	1	25
	Right	4	0	8	5	2	5	4	1	7	3	0	9	2	1	9
Lefts	Left	13	1	22	21	2	13	21	5	10	4	3	29	3	1	32
	Right	2	3	8	9	1	3	8	1	4	4	0	9	3	0	10
On visual field task																
Males	Left	10	5	18	22	1	10	18	5	10	7	2	24	4	1	28
	Right	4	0	8	7	0	5	8	0	4	2	0	10	3	0	9
Females	Left	7	1	21	10	5	14	13	3	13	6	2	21	6	1	22
	Right	4	4	12	11	3	6	13	0	7	2	0	18	1	0	19
Rights	Left	7	2	19	12	4	12	12	2	14	8	1	19	5	1	22
	Right	3	3	10	8	1	7	10	0	6	1	0	15	3	0	13
Lefts	Left	10	4	20	20	2	12	19	6	9	5	3	26	5	1	28
	Right	5	1	10	10	2	4	11	0	5	3	0	13	1	0	15

*< 0 05

(Table 3). In contrast, the frontal lobe areas were distributed more evenly in males and left-handers, showing slightly larger left frontal lobe areas in left dominant males and left-handers, and more equal, even the opposite distribution in right dominant individuals, although these differences did not reach significance. Females and right-handers with left dominance had significantly more frequent R > L frontal lobes, but the left side was significantly larger in females with right hemispheric dominance on the dichotic listening tasks ($\chi^2 = 5.6$, $P = 0.02$). A more even distribution of frontal lobe asymmetries was observed between left and right dominant individuals on the visual field preference task. The temporal lobe asymmetries were quite even with no significant difference between cerebral dominance, handedness or sex groups. Parietal lobe asymmetries were also evenly distributed with the exception of visually left dominant left-handers and females who had a higher frequency of larger left side.

Functional laterality groups and anatomical measures

When cerebral dominance was examined as a main effect, without considering the influence of sex or handedness, the anatomical measures differed only between the hand performance dominance groups. The main effect of hand dominance on linear measures $F(8,87) = 4.32$, $P = 0.0001$, and on area measures $F(8,90) = 2.28$, $P = 0.029$, was significant. Table 4 presents a summary of linear and area measures by hand performance. Univariate F analyses showed that the right anterior frontal, left and right parietal, and left occipital widths were significantly larger in left dominant individuals. Left dominant subjects also had larger left and right hemispheric areas, and larger right frontal and temporal areas, but this was only a trend for the lobar areas (Table 4). No significant

TABLE 3. AREA ASYMMETRIES AND CEREBRAL DOMINANCE

Subject group	*Cerebral dominance*	*Hemispheres*			*Frontal lobes*			*Temporal lobes*			*Parietal lobes*		
		L > R	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*	*L > R*	*L = R*	*R > L*
Dichotic listening													
Males	Left	3	0	30*	15	1	13	17	0	15	16	0	15
	Right	6	0	10	7	0	10	7	2	8	7	0	9
Females	Left	9	0	30	16	1	21*	20	1	18	23	1	15
	Right	0	0	10	7	2	1	7	0	3	6	1	3
Rights	Left	6	0	29	11	1	19	17	0	17	16	0	18
	Right	3	0	10	7	2	5	8	0	6	6	1	7
Lefts	Left	6	0	31	20	1	15	20	1	16	23	1	12
	Right	3	0	10	7	0	6	6	2	5	7	0	5
Visual field task													
Males	Left	6	0	28	16	1	16	15	2	17	16	0	16
	Right	3	0	10	5	0	7	7	0	6	7	0	6
Females	Left	8	0	23	14	3	13	16	1	14	19	1	11
	Right	3	0	18	10	0	10	11	0	10	12	1	8
Rights	Left	6	0	24	10	3	16	14	0	16	15	0	15
	Right	4	0	14	7	0	10	10	0	8	7	1	10
Lefts	Left	8	0	27	20	1	13	17	3	15	20	1	12
	Right	2	0	14	8	0	7	8	0	8	12	0	4

* < 0.05

TABLE 4 HAND PERFORMANCE AND ANATOMICAL ASYMMETRIES

	Dominance				
	Left $\bar{x}$	*(n = 51)* *SD*	*Right* $\bar{x}$	*(n = 52)* *SD*	*MANOVA* *P**
Linear					
L anterior frontal	43.82	(3.43)	43.85	(3.43)	0.935
R anterior frontal	43.97	(2.85)	42.81	(2.48)	0.021*
L posterior frontal	56.33	(3.71)	56.99	(3.23)	0.391
R posterior frontal	56.83	(3.40)	56.36	(3.16)	0.424
L parietal	63.25	(3.48)	60.67	(3.27)	0.0001*
R parietal	64.69	(3.23)	63.28	(2.84)	0.009*
L occipital	44.10	(3.63)	40.58	(4.23)	0.0001*
R occipital	46.06	(3.56)	45.31	(3.94)	0.244
Area					
L hemisphere	85.99	(7.59)	83.29	(8.12)	0.039*
R hemisphere	88.00	(7.26)	85.01	(7.63)	0.016*
L frontal	31.00	(2.36)	30.85	(3.05)	0.704
R frontal	31.10	(2.22)	30.34	(2.56)	0.064
L temporal	13.25	(1.56)	12.96	(1.65)	0.315
R temporal	13.28	(1.90)	12.73	(1.46)	0.076
L parietal	23.85	(2.13)	23.39	(2.08)	0.222
R parietal	23.38	(1.81)	23.31	(2.11)	0.160

*Significant univariate Fs.

differences were found between the dichotic listening or visual field dominance groups, and the results are not displayed. Correlations between continuous measures of laterality and anatomy revealed similar findings and will not be reported separately.

Hand performance and asymmetry ratios

The degree of asymmetry was also analysed by ratios of right and left measures, using the formula L−R/L+R for each anatomical structure. Using the ratios eliminates the influence of actual structure size, and measures the degree of asymmetry. Negative ratios reflect larger right side, positive ratios larger left side. Only the linear ratios were significantly different and only between the hand performance groups. Greater right than left anterior and posterior frontal ratios were observed in left dominant, and greater left than right ratios in right dominant subjects for hand performance. The degree of the occipital asymmetry ratio, showing a larger right side, was greater in the right dominant group (Table 5).

TABLE 5. LINEAR MEASUREMENT RATIO AND HAND PERFORMANCE

	Dominance				
	Left	*(n = 45)*	*Right*	*(n = 48)*	*MANOVA*
Measures	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*P**
Anterior frontal	−0.0027	(0.033)	0.0102	(0.027)	0.048*
Posterior frontal	−0.007	(0.021)	0.0046	(0.015)	0.003*
Parietal	−0.0108	(0.016)	−0.0176	(0.019)	0.062
Occipital	−0.0210	(0.041)	−0.0533	(0.046)	0.0001*

*Significant univariate Fs

Dichotic listening and anatomical asymmetries

(a) *Dichotic listening laterality and handedness interaction.* Significant interactions between auditory task dominance and handedness on linear anatomical measures [F(8,78) = 3.13, $P = 0.004$] and for area measures [F(8,82) = 2.56, $P = 0.015$] were observed on MANOVA. Table 6 summarizes the means and the significance of univariate Fs for each anatomical measure. Student-Newman-Keuls *post hoc* comparisons indicated the significant differences between the four group means for each anatomical measure. Left dominant right-handed subjects had larger right anterior frontal, left parietal and occipital widths and right frontal area than left-handers. Right dominant right-handers had larger left occipital width than left dominant left-handers.

The significant interactions are illustrated in Figs 3−6. They appear to follow the same direction with one exception. Among right-handers the left dominant individuals had larger linear and area measures and in left-handers the reverse was true: the right dominant group had larger anatomical measures. The exception was that both right and left dominant right-handers had larger left occipital width measure compared with left-handers (Fig. 6).

(b) *Dichotic listening laterality and sex interaction.* The interaction between auditory task laterality and sex [F(8,82) = 2.95, $P = 0.006$] is mostly related to the significant sex differences between anatomical measures, which is to be expected with generally larger brain size in males. However, on the right and left temporal area measures, the

TABLE 6 DICHOTIC LATERALITY BY HAND INTERACTION

		Laterality				
		L dom		R dom		
Measures	*Hand*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*Univariate F Significance P*
Linear	n = right	34		12		
	left	36		11		
R anterior frontal width	R	a 44.50	(2.93)	b 42.79	(2.08) *	0.006
	L	c 42.72	(2.54)	d 43.23	(2.52)	
L parietal width	R	a 63.65	(3.26)	b 62.31	(4.09)*	0.0001
	L	c 60.31	(3.07)	d 62.68	(2.63)	
L parietal width	R	65.01	(3.18)	63.92	(3.46)	0.010
	L	63.03	(3.18)	64.00	(1.95)	
L occipital width	R	a 44.34	(3.63)	b 43.67	(3.75)**	0.001
	L	c 40.15	(4.21)	d 41.73	(3.39)	
Areas	n = right	35		13		
	left	37		11		
R frontal area	R	a 31.28	(2.28)	b 30.49	(2.08)*	0.003
	L	c 29.93	(2.59)	d 31.71	(2.39)	
L temporal area	R	13.53	(1.57)	12.35	(1.34)	0.021
	L	12.93	(1.65)	13.43	(1.70)	
R temporal area	R	13.58	(2.00)	12.27	(1.43)	0.012
	L	12.71	(1.40)	12.90	(1.59)	
L parietal area	R	24.11	(2.23)	23.00	(1.69)	0.045
	L	23.35	(2.11)	23.85	(2.05)	
R parietal area	R	23.97	(1.99)	23.16	(1.11)	0.022
	L	23.10	(2.22)	23.81	(1.83)	
L hemispheric area	R	86.83	(7.54)	84.67	(8.41)	0.023
	L	82.63	(8.59)	85.84	(7.03)	
R hemispheric area	R	88.64	(7.60)	87.16	(7.04)	0.027
	L	84.49	(8.33)	87.12	(5.67)	

*Significant at $P < 0.05$ on Newman-Keuls between a and c; **significant at $P < 0.05$ on Newman-Keuls between a and c, b and c

right dominant females had larger or equal measures to right dominant males (Table 7). The only measures showing significant auditory laterality effect within the sex group were in the right temporal and right hemispheric areas: left dichotic dominant males had larger measures than right dominant males. Other interactions also followed the same direction. The anatomical measures were always larger in right dominant rather than left dominant females. The reverse appeared to be the rule for the males: larger measures in left dominant compared with right dominant males (Table 7). Males with left dominance for dichotic listening had significantly larger measures from all other groups (Fig. 7).

(c) *Dichotic listening laterality, sex and handedness interaction.* A significant three-way interaction between dichotic task laterality, handedness and sex was found for the parietal area ratios [F(4,77) = 3.61, $P = 0.009$]. Left dominant, left-handed females and right-handed males had larger left parietal areas. Right dominant, female right-handers and male left-handers had symmetrical left parietal areas. The parietal ratios showed larger right parietal areas in left dominant, female right-handers and male left-handers.

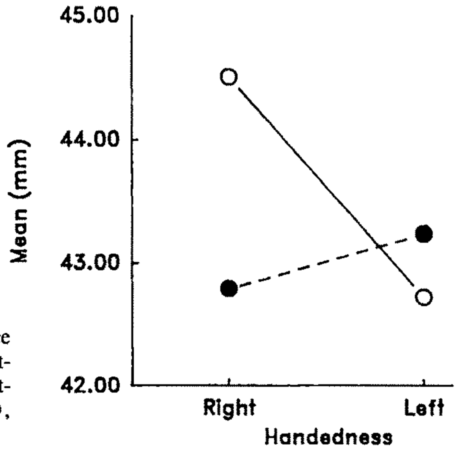

FIG. 3. Right anterior frontal width. Hand×Cerebral Dominance (dichotic task) interaction The difference between right- and left-handers is only significant among left dominant individuals. Right-handers have larger measures Dominance on dichotic listening: ○, left; ●, right

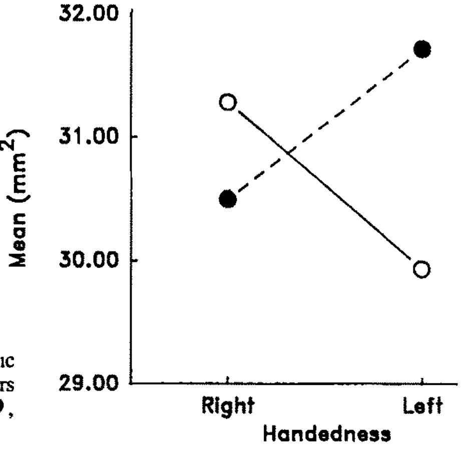

FIG. 4. Right frontal area. Hand×Cerebral Dominance (dichotic task) interaction Significant in the left dominant group Right-handers have larger areas Dominance on dichotic listening: ○, left, ●, right.

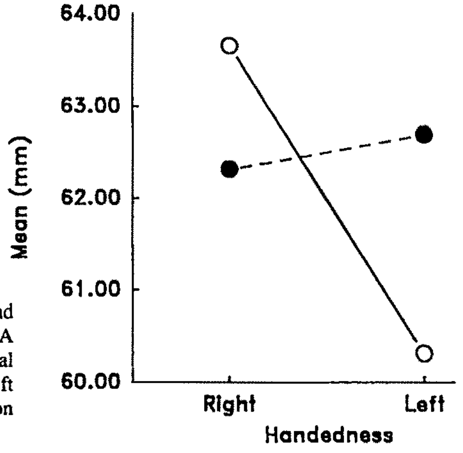

FIG. 5. Left parietal width. Interaction between handedness and cerebral dominance for dichotic listening: ○, left; ●, right. A reversal of the relationship between right- and left-handers is typical of all the anatomical measures. The difference between left hemisphere dominant, right- and left-handers is significant on Newman-Keuls.

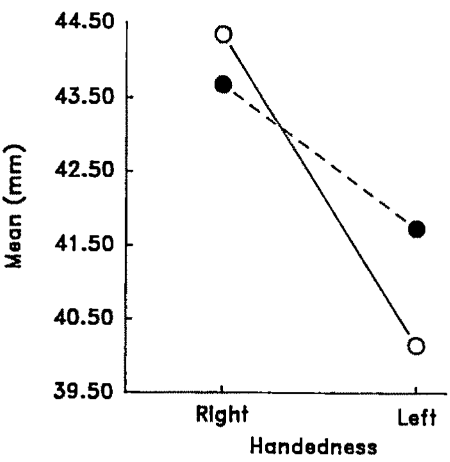

FIG. 6. Left occipital width Hand×Dichotic Listening Dominance. Both left and right dominant right-handers have larger measures than left dominant left-handers on Newman-Keuls Dominance on dichotic listening. ○, left, ●, right.

TABLE 7 DICHOTIC LATERALITY BY SEX INTERACTION

Measures	Sex	Laterality: L dom. $\bar{x}$	L dom. SD	R dom. $\bar{x}$	R dom. SD	Univariate F Significance P
Areas	F n	38		10		
	M n	33		14		
R frontal	F	a 29.43	(2.46)	b 30.66	(1.93)**	0.0001
	M	c 31.98	(1.79)	d 31.27	(2.49)	
L frontal	F	d 29.56	(2.82)	b 31.22	(2.00)*	0.0001
	M	c 32.21	(2.00)	d 31.33	(3.32)	
R temporal	F	a 12.55	(1.32)	b 12.69	(1.50)***	0.002
	M	c 13.85	(1.98)	d 12.46	(1.56)	
L temporal	F	a 12.74	(1.16)	b 13.08	(1.27)*	0.005
	M	c 13.81	(1.91)	d 12.67	(1.77)	
R parietal	F	a 22.63	(2.09)	b 23.09	(0.91)*	0.0001
	M	c 24.63	(1.66)	d 23.68	(1.74)	
L parietal	F	a 22.96	(2.18)	b 23.08	(1.73)*	0.006
	M	c 24.65	(1.83)	d 23.58	(1.99)	
R hemisphere	F	a 82.53	(7.53)	b 84.48	(4.97)**	0.0001
	M	c 91.31	(6.19)	d 89.04	(6.64)	
L hemisphere	F	a 80.75	(7.63)	b 81.85	(6.03)**	0.001
	M	c 89.41	(6.49)	d 87.60	(8.00)	

*Significant at < 0.5 Newman-Keuls between a and c; **significant at < 0.5 Newman-Keuls between a and c, a and d, ***significant at <0.5 Newman-Keuls between a and c, b and c, c and d

Visual dominance and anatomical asymmetries

(a) *Visual field laterality and handedness interaction.* The overall MANOVA between left and right hemispheric dominance on this task showed a trend towards significance on linear measures [F(8,75) = 1.63, P = 0.131]. The left parietal width interacted significantly with handedness and was found to be larger in left dominant individuals who were right-handed [Univariate F (P = 0.005)]. On the visual task, left-handers

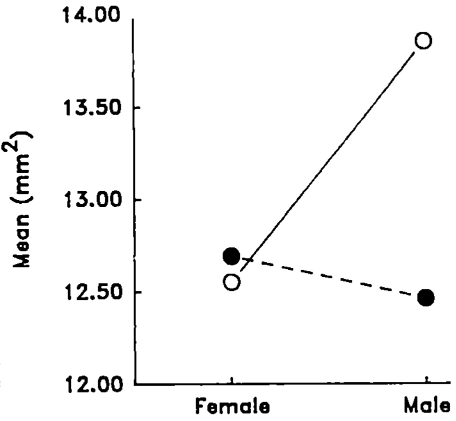

FIG. 7 Right temporal area. Sex and cerebral dominance on dichotic listening ○, left, ●, right Left dominant males have significantly larger measures from all the other groups.

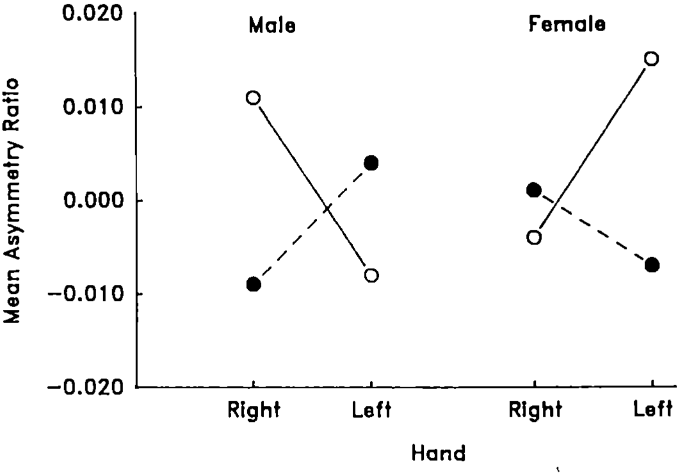

FIG. 8. Parietal area ratios. Hand × Sex × Cerebral dominance (dichotic task) interaction Mean area ratio measurements (L−R/L+R) are plotted or left and right dominant subject groups who are left- or right-handed, separately for males and females. In both gender groups the significant interaction occurs in left dominant individuals but the magnitude differs in the opposite direction among right- and left-handers (*see* text).

and right dominant individuals had smaller left parietal ($P = 0.005$), right parietal ($P = 0.03$) and left occipital ($P = 0.02$) width measures.

(b) *Visual field laterality by sex interaction.* The overall interaction did not reach significance ($P = 0.347$) on the MANOVA. However, the individual differences were all in the direction of larger structures in males than in females, which was expected, and will not be detailed further. The overall direction of interaction between left and right dominance within the sexes was interesting and reflected a similarity with the dichotic listening results. The left dominant males had larger structures than right dominant males, with the exception of the left occipital width which was about equal. The female dominance groups were much closer although there was a trend towards slightly larger structures in left dominant females as well.

DISCUSSION

Our ability to measure anatomical asymmetries *in vivo* provides a unique opportunity for correlation with functional laterality. The correlation proved to be rather complex, as multiple factors seem to interact. In our study the structural measures showed the strongest and most consistent differences between the cerebral dominance groups for hand performance. Left hemisphere dominance on this task was associated with larger right frontal and left occipital widths similar to previous CT studies (LeMay and Kido, 1978). When cerebral dominance was determined by the dichotic listening paradigm, the differences were only significant when interactions between dichotic listening laterality, handedness and sex were considered.

The examination of the dichotic listening and handedness interaction uncovered a uniquely consistent direction. Subjects in whom the same hemisphere is dominant for both handedness and language have larger structures than those in whom dominance is crossed. The consistency throughout all the structures supports the validity of these findings and suggests that the coincidence of certain functional lateralities are associated with larger structures. Anatomical measures were found to be less significantly different among visual field laterality groups. However, left visual field dominance for reading, interacting with right-handedness, was associated with wider left parietal width.

The anatomical measures were selected because of known relevance to auditory language (temporal or parietal lobe cross-sectional areas, parietal and posterior frontal widths) or visual language (parietal lobe area, parietal and occipital widths). In absolute measures, the right hemisphere and many of the right-sided anatomical structures were larger, supporting some other studies reviewed in the Introduction. Left dominance for auditory and visual task was more frequent as expected but instead of larger structures on the left in individuals with left-sided dominance and the reverse in right-sided dominance, the asymmetries were related to a complex interaction of handedness, sex and task dominance. The linear and area measures were complementary although they sampled somewhat different areas of the brain. A three-dimensional rostrocaudal 'torque' was postulated where upper and rostral portions of the hemispheres appeared larger on the right and the lower and caudal portion slightly larger or equal on the left (Kertesz *et al.*, 1990). This could be the reason why in certain CT studies using a lower axial cut, larger left hemisphere measures were recorded (Yeo *et al.*, 1987).

The direction of asymmetries shows differences in the various dominance groups. The more prevalent left dominant males show a definite R > L asymmetry of sagittal length and hemispheric area in contrast to the atypically right dominant males who show a more even distribution. The frontal linear asymmetries vary with sex and cerebral dominance to a greater extent than the posterior head regions, in which a larger right side is shown, irrespective of cerebral dominance. Lobar area asymmetries were more evenly distributed, with the exception of the frontal lobes. When cerebral dominance for dichotic listening and handedness is taken into consideration, there is a significantly larger right frontal lobe area in left dominant females and in all right-handers. This would correspond to the more typical right frontal petalia on the CT scan. It is interesting that left-handers have a larger left frontal area, regardless of cerebral dominance, and the atypically right dominant females and right dominant right-handers join the left-handers in having a larger left frontal area. Sex and handedness interaction with dichotic listening appears to be in the opposite direction, as evidenced by the contrasting association of males and left-handers and females and right-handers in the anterior frontal width asymmetry discussed above. This is compatible with the direction of dissociations postulated by Galaburda *et al.* (1990).

That right-handers have larger right, and left-handers have larger left frontal lobes is contrary to the expectation of a larger right frontal lobe to be associated with superior motor skills in the left hand. This finding is, however, compatible with the CT scan studies which associate larger right frontal petalia with right-handers and less asymmetry or atypical L > R distribution of the 'petalia' with left-handers. Petalia measures are of course different from the frontal width or frontal cross-section areas used in this study, but the similarity in the direction of asymmetries supports the validity of our observations. The biological significance of the right frontal petalia in right-handers is not clear but theoretically it has been considered to be a complementary enlargement opposite to the larger left posterior language related structure (LeMay and Kido, 1978). Our measures, however, indicate no overall left-sided posterior prominence but a much more complex relationship between posterior structures, handedness and functional laterality. The continuous variables of task laterality and the degree of asymmetry were analysed with canonical correlations and multiple repression analysis. The findings were less contributory or similar to the multivariate comparisons based on dichotonous dominance groups and were not reported separately in the interest of space.

The multi-dimensional associations highlight the complexity of the relationship between functional and anatomical laterality, and may provide a basis to explain some individual differences in cerebral organization, behaviour and recovery from brain damage. Sex and handedness are important to a certain extent but there are a multitude of other factors contributing to the structural and functional diversity of the brain. Genetic factors in anatomical variability have been suggested (Galaburda *et al.*, 1990) and there is experimental evidence concerning environmental influences (Rakic and Williams, 1986). Some asymmetries have been postulated to be related to hormonal effects on intra-uterine development such as testosterone retarding the growth of the left side of the brain (Geschwind and Galaburda, 1985). Recent observations, however, suggest that the left planum temporale seems to be rather constant in size, while variations in the size of the right side result in the asymmetry (Galaburda *et al.*, 1987).

The most obvious biological advantage of hemispheric specialization is the enhanced ability of a complex structure to handle complex tasks. A purely symmetrical structure is more likely to represent a duplication of functions. Asymmetrical representation in the brain allows a diversity of complementary functions permitting greater adaptability. It has been held that the further an organism advances on the phylogenetic scale, the more functional and anatomical asymmetries can be demonstrated in the nervous system. Although attempts have been made to associate left parietal petalia with the development of language, phylogenetically, our studies caution against such an obviously straightforward relationship. Recently, a surprising amount of cerebral asymmetry has been shown in many species throughout the phylogenetic scale. There is considerable evidence of anatomical asymmetry in animals that is not related to language (Glick, 1985).

For a while it was accepted that functional asymmetries can occur without any obvious anatomical ones. However, the relationships of anatomical asymmetries to functional ones are of great theoretical interest. In addition to the theoretical issues there is a practical and clinical relevance to imaging certain asymmetries which may have a predictable association with language or with other cognitive functions. For instance, invasive tests of cerebral dominance for language, such as the WADA test during angiograms, could be supplanted by non-invasive imaging of anatomical symmetries if reliable associations could be demonstrated. So far this has not occurred. Another potential clinical use of imageable cerebral asymmetry is the possible prediction of recovery from language deficit after stroke or other acute recoverable cerebral insult. If cerebral function is more symmetrically distributed, and this is associated with anatomical symmetry, then recovery may be faster or more complete or both, because the homologous structures may have more compensatory capacity (Pieniadz *et al.*, 1983). These pilot findings have not been confirmed by other studies (Kertesz, 1988). Anatomical asymmetries that are systematic may be important in stereotactic surgery where the difference of a few millimetres can influence functional outcomes. Metabolic imaging also requires increasing accuracy in anatomical localization for the interpretation of physiological data. Cerebral morphometric techniques will be essential for many areas of neurology and neuroimaging in the development of diagnostic and clinical tools and the assessment of cerebral function.

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THE PLASTICITY OF CUTANEOUS HYPERALGESIA DURING SYMPATHETIC GANGLION BLOCKADE IN PATIENTS WITH NEUROPATHIC PAIN

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SUMMARY

In order to investigate the plasticity of cutaneous sensory abnormalities in neuropathic pain, we monitored sensory and vasomotor effects of diagnostic sympathetic ganglion blocks in 24 patients, who suffered from chronic pain and cutaneous hyperalgesia following peripheral nerve or tissue injury. Ongoing pain was rated on a visual analogue scale, and pain evoked by innocuous tactile and cooling stimuli (hyperalgesia) on a verbal rating scale. Skin temperatures were determined at symmetric sites. In two patients, cutaneous blood flow was measured with a laser Doppler device.

The sympathetic blocks led to a significant reduction of the group mean ongoing pain (40%) and cutaneous hyperalgesia (50%). Between patients, however, there was a large variability that could not be related merely to adequacy of sympathetic blockade. Neither the magnitude of change in skin temperature nor the final skin temperature after the block correlated with the amount of pain relief. The relief of hyperalgesia, however, correlated with the relief of ongoing pain.

Nine patients experienced pain relief of greater than 50%. In these patients, the time course of hyperalgesia relief was similar to the time course of relief of ongoing pain. Pain relief occurred simultaneously with or a few minutes before cutaneous vasodilatation. During the block, even vigorous mechanical or cold stimuli did not rekindle hyperalgesia. In all patients, pain and hyperalgesia returned within a day after the block. In three patients tested, passive warming of the limb to the temperature achieved by the sympathetic block had negligible effects on pain and hyperalgesia.

The hyperalgesia of sympathetically maintained pain is thought to be due to sensitization of central pain-signalling neurons to mechanoreceptor input. The present data indicate that this sensitization is highly plastic even when the disease has persisted for months or years. It could be reversed within minutes by a sympathetic blockade, but returned when sympathetic block subsided. Mechanoreceptor input by itself was not sufficient to maintain or rekindle the central sensitization. This supports the hypothesis that low-grade activity of nociceptors, possibly due to development of α-adrenergic sensitivity after injury, is involved in the maintenance of central sensitization.

INTRODUCTION

Peripheral nerve injury and soft-tissue injury can both lead to disabling chronic pain. This chronic pain is typically accompanied by hyperalgesia to mechanical stimuli (Loh and Nathan, 1978; Lindblom and Verrillo, 1979; Frost *et al.*, 1988). In many patients, the mechanical hyperalgesia is such that stroking the skin with a soft brush, applying a vibrating tuning-fork to a bony prominence near the border of the hyperalgesic zone,

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or movement of a single hair shaft can cause pain. This striking response to innocuous stimuli is sometimes referred to as allodynia (Merskey, 1986).

The hyperalgesia to light touch in many patients is due to altered neural coding such that the excitation of low-threshold mechanoreceptors can lead to painful sensations. This has been demonstrated by reaction times (Lindblom and Verrillo, 1979; Campbell *et al.*, 1988*b*), preferential A-fibre block by ischaemia or pressure (Wallin *et al.*, 1976; Campbell *et al.*, 1988*b*) and electrical stimulation at $A\beta$-fibre strength (Price *et al.*, 1989; Treede *et al.*, 1991). The observation that mechanoreceptors rather than nociceptors may be responsible for the touch-evoked pain indicates that changes in central neurons must occur (Loh and Nathan, 1978; Tahmoush, 1981). In other words, central neurons, whose activity leads to pain, develop responsiveness to non-nociceptive input, e.g. from low-threshold mechanoreceptors (central sensitization). There is experimental evidence that these changes can be initiated by the afferent barrage associated with the initial injury (e.g. Cook *et al.*, 1987; Laird and Cervero, 1989; Simone *et al.*, 1989). Little is known, however, concerning the processes responsible for maintaining the central sensitization in a chronic state.

A common feature of some pain syndromes such as causalgia and reflex sympathetic dystrophy is the relief obtained from sympathetic ganglion blockade (Bonica, 1979). This common feature has led to the introduction of the term 'sympathetically maintained pain' (SMP) to encompass those chronic pain syndromes that can be relieved by sympathetic blocks (Roberts, 1986). Chronic pain with similar clinical characteristics that is not relieved by sympathetic blocks is referred to as sympathetically independent pain (SIP) (Campbell *et al.*, 1988*a*). Since both SMP and SIP can be the sequelae of damage to the nervous system, both are often referred to as neuropathic pain. 'Pain after peripheral injury' is an alternative term that emphasizes the fact that often damage to major nerves cannot be demonstrated in either SMP or SIP (Devor *et al.*, 1991). The role of damage to small nerve branches in soft-tissue injury that leads to SMP or SIP has not been determined. The appropriate terminology in this field is therefore still a matter of debate.

A well-known model for SMP (Roberts, 1986) stipulates that the central sensitization in SMP is maintained by ongoing activity of mechanoreceptors that are excited by sympathetic efferents. Alternatively, low-grade activity of nociceptors may be necessary to maintain a chronic central sensitization (Campbell *et al.*, 1988*a*). The aim of the present study was to gain insight into the presumed mechanisms of central sensitization occurring in SMP. For this purpose we determined (i) the time course of pain relief following sympathetic ganglion blocks; (ii) whether the relief of hyperalgesia parallels that of ongoing pain; (iii) whether mechanoreceptor excitation during sympathetic blockade is sufficient to rekindle pain and hyperalgesia; and (iv) how quickly pain returns after the block subsides. For comparison, we monitored skin temperature as an index of the blockade of the sympathetic innervation of cutaneous blood vessels.

METHODS

The study was performed in 24 patients who were admitted to our outpatient pain treatment centre because of chronic non-malignant pain in an extremity and were suspected of having SMP. The study protocol was approved by the institutional Clinical Investigations Committee, and informed consent was obtained from each patient. Table 1 summarizes the clinical data of these patients. At the time of the study, symptoms

TABLE 1. CLINICAL DATA FROM THE MEDICAL RECORDS OF 24 NEUROPATHIC PAIN PATIENTS

Case	*Age (yrs)*	*Sex*	*Duration of symptoms (yrs)*	*Type of injury*	*Burning pain*	*MH*	*CH*	*Pain relief (%)*
Lumbar sympathetic blocks								
1	20	F	2.5	Fracture of a sesamoid bone	+	+	+	97
2	29	F	3	Sciatic nerve injury during hip surgery	−	+	+	95
3	52	F	12	Sural nerve neuroma	*	+	+	69
4	31	F	4	Torn ligaments, ankle surgery	+	+	+	69
5	38	F	0.5	Lumbar disc surgery, arachnoiditis	−	+	+	57
6	56	F	4	Torn ligaments, ankle surgery	*	+	−	33
7	28	F	2.5	Blunt knee injury	−	+	+	33
8	32	M	4	Peroneal nerve entrapment	−	+	+	24
9	20	F	2	Sprained foot	+	+	+	17
10	58	M	3	Hip fracture, sciatic nerve crush	*	−	−	13
11	34	M	3	Lumbar fracture, tarsal tunnel	*	+	+	12
12	57	F	0.5	Bunion surgery	+	+	−	9
13	35	F	6	Foot injury, tarsal tunnel	+	+	+	6
14	44	M	1.5	Gunshot wound, sciatic nerve injury	+	+	+	0
15	60	M	3	Revascularization surgery	*	+	n.t.	0
Cervico-thoracic blocks								
16	39	F	3	Car accident, root and plexus injury	+	+	+	100
17	54	M	1.5	Wrist hyperextension injury	−	+	+	78
18	35	F	3	Mild crush injury, trigger finger release	*	+	+	67
19	34	M	5	Sprained wrist, 5th metacarpal fracture	+	+	+	67
20	37	M	2	Frozen shoulder, thoracic outlet syndrome	*	−	−	42
21	40	F	4	Blunt trauma between scapulae	*	+	+	35
22	40	M	2	Crush injury of fingers, amputation	*	+	+	30
23	50	M	6.5	Hand injury, carpal tunnel	*	+	+	23
24	38	F	2	Wrist hyperextension injury	+	+	+	0

MH = hyperalgesia to mechanical stimuli; CH = hyperalgesia to cold stimuli, +/− = symptom present/absent, n.t. = not tested, *not formally tested by McGill Pain Questionnaire.

had been present for 6 mths to 12 yrs. The pain initially was precipitated by nerve injury (n = 7), bone fracture (n = 3), soft tissue injury (n = 10), or surgery (n = 4). All patients suffered from chronic, continuous pain. The quality of pain was assessed in 14 patients using the McGill Pain Questionnaire (Melzack, 1975). There was no difference in pain relief from sympathetic blockade between those nine patients who marked burning pain and those who did not. In 22 patients, light mechanical stimuli were painful (mechanical hyperalgesia); 19 patients also reported hyperalgesia to cooling-stimuli. In 13 patients, the pain threshold on the affected side was as low as the detection threshold for mechanical stimuli on the control side. In five other patients, pain and detection thresholds on the affected side were the same, but both were higher than the normal detection threshold.

Patients underwent either a lumbar sympathetic ganglion block (LSB, n = 15) or a cervico-thoracic sympathetic ganglion block (CSB, n = 9), depending on pain location. All blocks were performed with 0.25% bupivacaine hydrochloride solutions. The LSBs were carried out under fluoroscopic guidance with the single needle technique described by Hatangdi and Boas (1985) using 15–20 ml of the local anaesthetic. The anterior paratracheal approach (LeRiche and Fontaine, 1934) was used for the CSB using 10 ml of the local anaesthetic. After the block, the patient's capacity to detect touch, cold and pin-pricks was determined to verify that none of the procedures caused a concomitant somatic block by diffusion of local anaesthetic to adjacent roots or dorsal root ganglia. Placebo injections were not performed, because patients would not tolerate a needle in the neck or back for longer periods of time, but 16 of the patients underwent a second sympathetic block using systemic administration of phentolamine, which was preceded by saline injections. This test identified two placebo responders, who are not included in the present study. The methods and results of the phentolamine blocks have been published elsewhere (Raja *et al.*, 1991).

Patients were asked to mark the intensity of their ongoing, stimulus-independent pain on a 100 mm visual analogue scale (VAS). The VAS was anchored at 0 (no pain) and 100 (most intense pain). Stimulus evoked pain was rated on a 0 (no pain) to 10 (most intense pain) verbal scale. For each patient, a few stimuli which induced pain in the affected area were selected from the following list: stroking with a soft brush, blunt pressure (force = 2 N, area = 133 mm^2), application of a drop of acetone that produces an ~2 °C drop in skin temperature (Frost *et al.*, 1988), contact with a brass probe drawn from an ice-water bath. These measurements were carried out every 5 min, for 10–30 min before the injection and for 15–30 min after the injection. Pain relief was computed from the two lowest consecutive ratings after the block and the mean of all ratings before the block. When necessary for data analysis, SMP was operationally defined as ongoing pain that was relieved by at least 50% following a sympathetic block. In four patients, the monitoring extended up to 10 h after the block on an hourly basis. In addition, all patients completed a diary about the effects and side-effects during recovery from the blocks.

Skin temperatures were measured at 1 min intervals using a surface temperature scanner (STS 100, Omega Medical, Florida, USA) and two thermocouples taped to the skin (YSI) at corresponding sites along the affected and the unaffected contralateral limb. The room temperature was 24 ± 1 °C (mean ± SD). In two patients undergoing LSB, cutaneous blood flow was also monitored directly with a laser Doppler device (Laserflo^{TM}, TSI Inc., St Paul, MN, USA), simultaneously with skin temperatures. In three patients, the affected limb was passively warmed to the temperature achieved by the sympathetic block. This was carried out on a separate occasion using either a heat lamp (n = 2) or a water bath (n = 1). Sensory testing and rating were performed as with the sympathetic block.

RESULTS

Example of a case with sympathetically maintained pain

A typical example of the changes in pain and in skin temperature during a sympathetic block is shown in Fig. 1. This patient (Case 16) suffered from constant burning pain in her left arm and touch-evoked pain in the same area. The patient's ratings of ongoing pain were stable over a period of 15 min prior to the block procedure. At time zero,

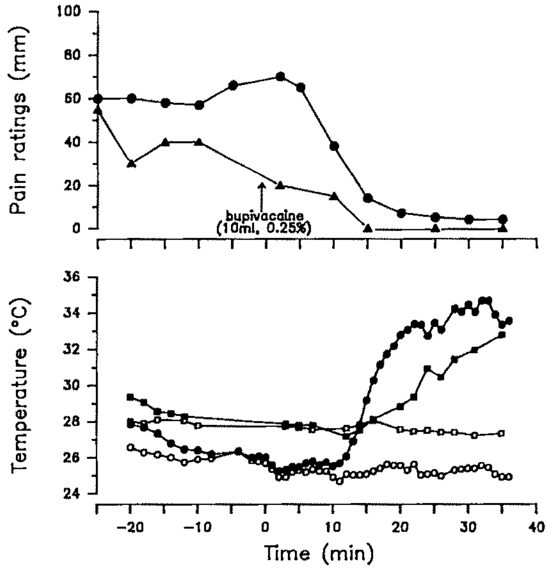

FIG. 1. Change in VAS ratings of ongoing pain (closed circles), hyperalgesia to brushing (closed triangles), skin temperature ipsilateral (closed symbols) and contralateral (open symbols) to an injection of 10 ml bupivacaine (0.25%) around the cervical sympathetic chain. Temperatures were measured at the tip of the 5th finger (circles) and the elbow (squares). Note the inversion of the proximal-distal temperature gradient in the arm ipsilateral to the injection. Concomitant with the temperature changes, this patient experienced a complete relief of her ongoing pain and her cutaneous hyperalgesia to mechanical stimuli.

10 ml bupivacaine were injected through a needle placed in the vicinity of the left cervical paravertebral ganglia. Within 10 min after the injection, pain was reduced to half of the pre-block value, and after 20 min the pain ratings were close to zero. Pain relief was nearly complete for 8 h after the block procedure. Pain evoked by light brushing (i.e. mechanical hyperalgesia) was reduced with a similar time course as the ongoing pain.

To compare cutaneous vasodilatation with the changes in ongoing pain and cutaneous hyperalgesia, skin temperatures were measured bilaterally at the tip of the 5th finger and at the dorsal hand. Before the block, temperatures at the distal sites were lower than at the proximal sites, both on the affected arm (closed symbols) and the unaffected right arm (open symbols). About 10 min after the injection, temperatures in the affected arm began to rise. The temperature change first appeared at distal sites, i.e. the fingertip. About 15 min after the injection, the proximal/distal temperature gradient was reversed, and the fingertip was warmer than the hand. Similar changes in temperature gradients were observed in all patients, where multiple sites were monitored (one other patient during CSB, six patients during LSB), and we therefore chose to monitor all skin temperatures at the most distal sites (fingertip or toe), independent of pain location.

Correlation of changes in ongoing pain, hyperalgesia and skin temperature

The mean initial ongoing pain was decreased from 59±24 mm (mean±SD) on the 100 mm visual analogue scale to 35±24 mm, a 41% decrease ($P \leq 0.001$, paired t test, $n = 24$). Between patients, however, there was a substantial variability in the amount of pain relief, ranging from complete relief to no relief at all. Mechanical hyperalgesia on the average was decreased by 47% and cold hyperalgesia by 52%. The relief of hyperalgesia was also variable between patients, but those patients who obtained good relief of ongoing pain also obtained good relief of hyperalgesia. This was shown by the significant correlation of the changes in mechanical hyperalgesia ($r = 0.88$, $P \leq 0.001$) and cold hyperalgesia ($r = 0.66$, $P \leq 0.05$) with changes in ongoing pain.

To test whether variability in pain relief was due to differences in individual pathology or due to differences in adequacy of the sympathetic blocks, the maximum change in ongoing pain in every patient was plotted as a function of the block effect on vascular innervation. Two parameters were used to estimate the extent of the sympathetic block: change in skin temperature (Fig. 2A) and the final temperature reached after the block (Fig. 2B). There was no correlation of pain relief with either measure of block adequacy ($r = -0.141$, $r = -0.087$). A final skin temperature between 32°C and 35°C at the distal limb probably indicates a near maximal sympathetic block. The change in skin temperature, however, was more variable and correlated inversely with the initial temperature (Fig. 2c, $r = 0.696$, $P \leq 0.001$). The data for both block locations (CSB, circles; LSB, squares) fell along the same regression line with a slope of -0.7 (Fig. 2c). The regression line indicates that 36°C is approximately the asymptotic temperature that can be reached in distal limbs after sympathetic blockade. The temperature data of four patients were far outside the regression line. These patients had a final skin temperature of less than 30°C (Fig. 2B), and one may thus suspect an incomplete sympathetic block. Two of these patients, however, experienced more than 50% pain relief, even though maximal sympathetic blockade was not achieved, and were classified as SMP (closed symbols in Fig. 2). We therefore conclude that incomplete sympathetic block did not play a significant role for the variability of pain relief between patients.

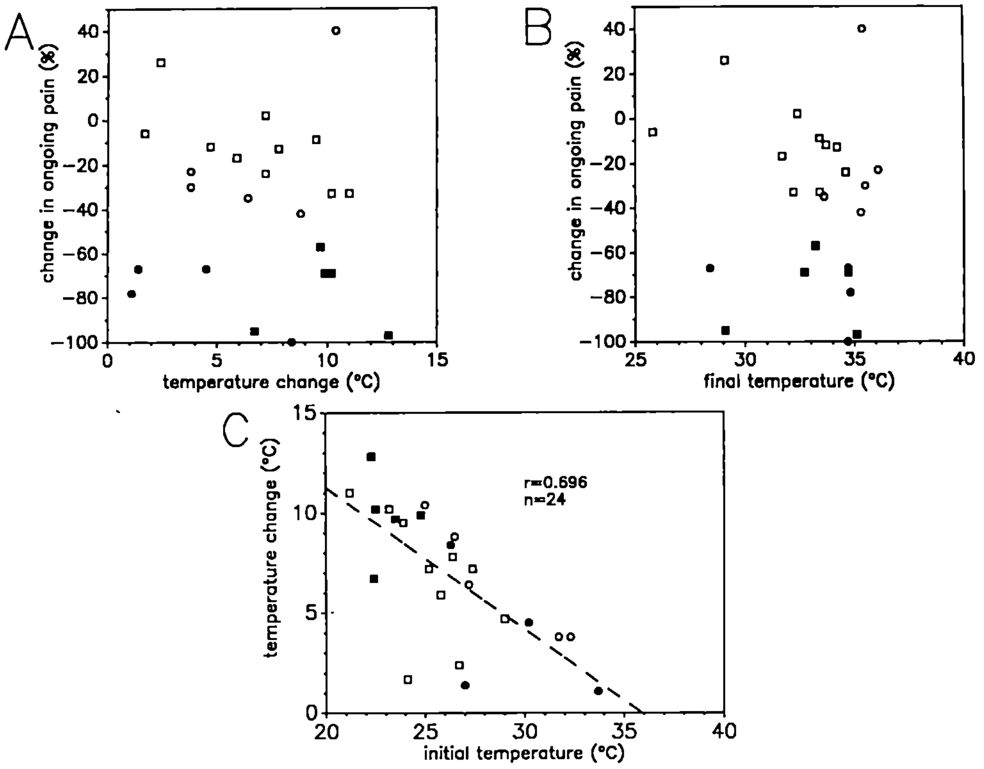

FIG 2. A, B, lack of correlation between pain relief and cutaneous vasodilatation. Changes in VAS ratings of ongoing pain are plotted as a function of cutaneous temperature change (A) or final temperature at the end of the sympathetic ganglion block (B). Pain relief did not correlate with any measure of block adequacy C, change in skin temperature after sympathetic ganglion blocks as a function of initial temperature The regression line intersects the x-axis at 36°C, indicating that this is probably the highest distal skin temperature that can be reached with complete cutaneous vasodilatation Squares, lumbar blocks; circles, cervico-thoracic blocks. Open symbols, SIP patients, closed symbols, SMP patients. Temperatures were measured at a distal site (fingertip or toe)

In three patients who had obtained good relief from a LSB, the affected limb was passively warmed to about the same temperature that was obtained during the block. Whereas the sympathetic block had on the average led to 80 ± 16% pain relief in these three patients (mean ± SEM), relief by passive warming was only 15 ± 4% ($P \leq 0.05$, paired t test). This indicates that pain relief in these patients was not merely due to an increase in skin temperature.

Time course of vasoconstrictor blockade

The time courses of distal skin temperature changes during the blocks were averaged to facilitate comparison with sensory effects. To determine if systematic differences between cervico-thoracic and lumbar sympathetic ganglion blocks occurred, the temperatures were first averaged separately for the two block locations (Fig. 3A). The base temperature in the fingertips was significantly higher than in the toes. (Table 2). Since the change in temperature was greater in LSB than in CSB, both blocks reached similar

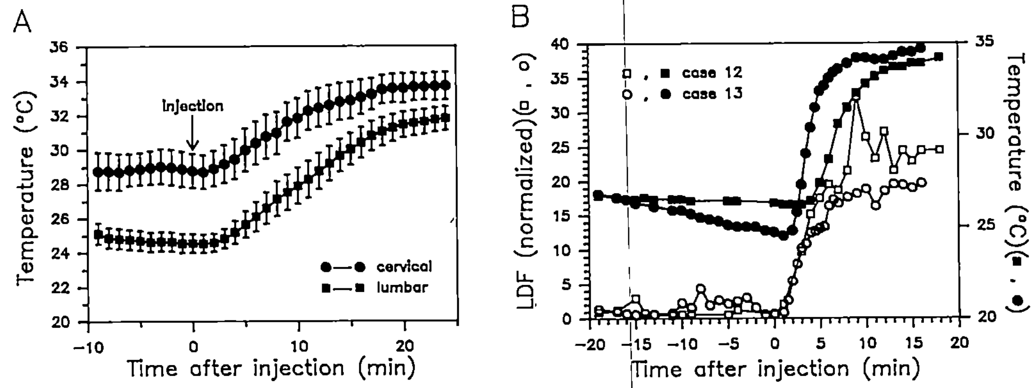

FIG. 3 A, time course of skin temperature changes after sympathetic ganglion blockade. Lumbar (LSB, n = 15) and cervico-thoracic blocks (CSB, n = 9) were pooled separately to show the difference in initial temperature Otherwise, the characteristics of both blocks were similar, consisting of a latency period, an initial fast rise, followed by a slower increase that extended beyond the observation period in four patients. B, comparison of skin temperature with a direct measure of cutaneous vasodilatation (laser Doppler). Measurements from two patients (open and closed symbols) after lumbar sympathetic block. The changes in laser doppler flow (LDF) (open symbols, left y-axis) preceded the temperature changes (closed symbols, right y-axis) by 1 min and 3 min, respectively.

TABLE 2 SKIN TEMPERATURE CHANGES FOLLOWING SYMPATHETIC GANGLION BLOCKS

	Lumbar blocks			*Cervico-thoracic blocks*			
	Mean	*SEM*	*n*	*Mean*	*SEM*	*n*	*P*
Affected side							
Before block (°C)	24.6	0.6	15	28.9	1.0	9	≤ 0.001
After block (°C)	32.4	0.7	15	34.3	0.8	9	n.s.
Difference (°C)	7.8	0.8	15	5.4	1.1	9	n.s.
$t_{1°C}$ (min)	8.2	1.1	15	6.8	1.6	9	n.s
$t_{90\%}$ (min).	16.9	1.8	15	16.7	2.9	9	n.s.
Unaffected side							
Before block (°C)	24.8	0.8	12	28.8	0.9	9	≤ 0.01
After block (°C)	24.3	0.6	12	30.5	1.5	9	≤ 0.001
Difference (°C)	−0.55	0.27	12	1.7	1.1	9	≤ 0.05

$t_{1°C}$ = time to reach 1°C temperature increase; $t_{90\%}$ = time to reach 90% of final temperature change, n.s. = $P > 0.05$, non-paired t test (LSB vs CSB)

final temperatures. For both locations, temperature increased by 1 °C within 7−8 min after the injection (range 2−16), and reached 90% of the final temperature change within about 17 min. This indicates that there was no qualitative difference between time courses of CSB and LSB, and that the final skin temperature after the block is a useful criterion for block adequacy.

Due to the thermal inertia of the limb, skin temperature may be a poor indicator of the time course of the sympathetic block. We therefore also measured the increase in cutaneous blood flow by using a laser Doppler device. The measurements in two patients undergoing a LSB (Fig. 3B) showed that the increase in cutaneous blood flow preceded distal skin temperature changes by only 1 min and 3 min, respectively. Temperature time courses at the toe thus provided a fairly accurate measure of peripheral vasodilatation.

Effects on contralateral skin temperature (i.e. the unaffected side) were sometimes observed in the CSBs, but not in the LSBs. In four patients, contralateral skin temperature after CSB increased by more than 2 °C. In two of them, the contralateral change was even larger and led to a higher final temperature than the ipsilateral change. As a consequence, the difference between finger temperature and toe temperature was more pronounced after the block than before the block (Table 2). Skin temperature decreased by 0.6 ± 0.3 °C in the LSBs and increased by 1.7 ± 1.1 °C in the CSBs. Although these changes were not significant versus baseline, there was a significant difference between CSB and LSB. These data are from 21 patients, because in one patient contralateral sites were not available due to a below-knee amputation, and in two other patients because of a previous lumbar sympathectomy on that side. The contralateral effects after cervico-thoracic ganglion block were a curious observation in this study. Cross-innervation or spread of the local anaesthetic within the same fascial space could account for this observation.

Relief of sympathetically maintained pain and hyperalgesia

To study the time course of pain and hyperalgesia relief we selected those nine patients who obtained 50% or more relief of their ongoing pain. These patients were considered to have predominantly sympathetically maintained pain (SMP). Table 3 compares the characteristics of these patients with those of the remaining patients that had predominantly sympathetically independent pain (SIP). There was no group difference with respect to initial pain intensity, initial skin temperature, or block adequacy. By definition, the pain ratings after the sympathetic block were significantly lower in SMP than in SIP. The same was true for mechanical hyperalgesia and cold hyperalgesia, underlining the correlation between ongoing pain and hyperalgesia. Those patients who were classified

TABLE 3. COMPARISON OF SYMPATHETICALLY MAINTAINED PAIN (SMP) AND SYMPATHETICALLY INDEPENDENT PAIN (SIP)

	SMP mean	*SEM*	*n*	*SIP mean*	*SEM*	*n*	*SMP vs SIP P*
Skin temperature before block							
Affected side (°C)	25.9	1.3	9	26.4	0.8	15	n.s
Affected − control (°C)	0.1	0.4	8	−0.3	0.4	13	n.s.
Skin temperature after block							
Affected side (°C)	33.0	0.9	9	33.1	0.7	15	n.s.
Pain and hyperalgesia							
VAS pre (mm)	65.3	6.0	9	55.5	6.9	15	n.s
VAS post (mm)	15.9	4.2	9	46.0	5.7	15	≤ 0.01
MH relief (%)	81	10	6	13	9	6	≤ 0.01
CH relief (%)	90	10	5	21	12	6	≤ 0.01
Ph relief (%)	76	7.1	8	19	9.6	8	≤ 0.01

VAS = visual analogue scale rating of ongoing pain; MH = hyperalgesia to mechanical stimuli, CH = hyperalgesia to cold stimuli; Ph = relief of ongoing pain by i.v. phentolamine block, SMP = > 50% relief of ongoing pain; SIP = < 50% relief of ongoing pain; n.s. = $P > 0.05$, non-paired *t* tests (SMP vs SIP) as having SMP also obtained significantly more pain relief from phentolamine blocks than those with SIP.

The initial skin temperatures were not lower in SMP than in SIP. This unexpected result might have been due to an unequal distribution of affected limbs. About half of the SMP patients had an affected area in the arm, where initial temperatures were generally higher than in the leg, but the arm was affected in only one-third of the patients with SIP. This imbalance could have masked a difference between SMP and SIP, but a two-way ANOVA failed to detect a significant main effect (F = 0.43) or interaction (F = 1.82). Analysis of the temperature difference between control and affected side also failed to detect any difference between SMP and SIP patients.

Figure 4 shows the time courses of the sensory changes and skin temperature in eight SMP patients following sympathetic blockade; in one SMP patient ratings had been obtained only once before and once after the block. The time to reach 50% of the final temperature increase (Fig. 4A) was 11.8 ± 2.5 min (mean ± SEM). The time to reach 50% of the final pain relief (Fig. 4B) was 7.9 ± 1.5 min (mean ± SEM). Pain relief thus

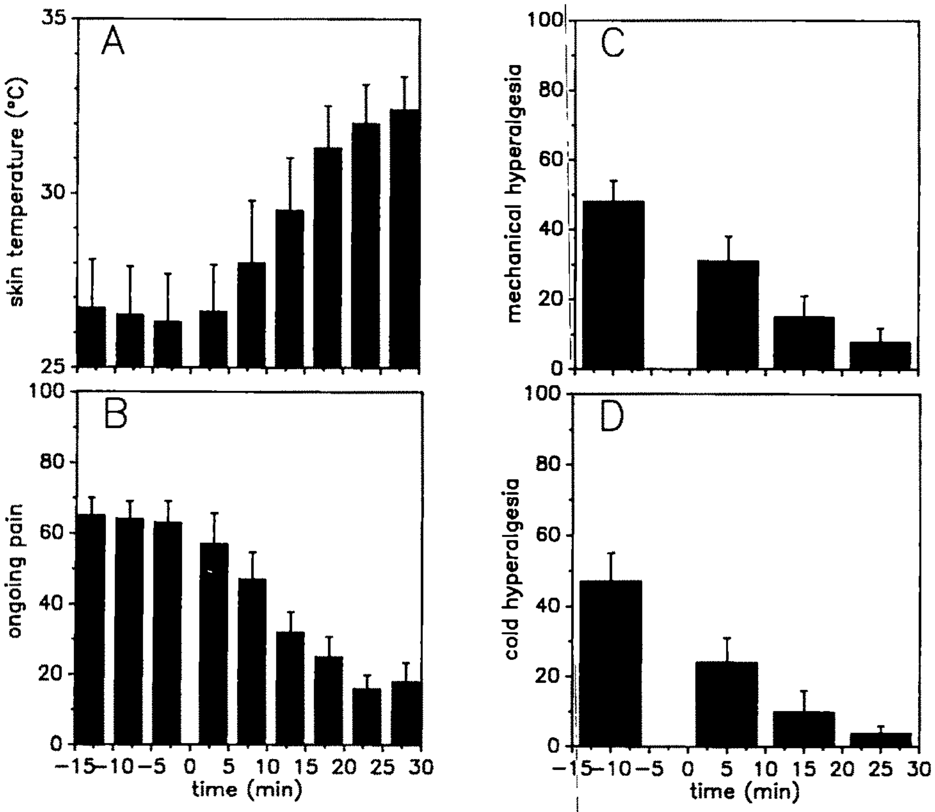

FIG. 4. Time course of mean change in skin temperature (A), visual analogue scale ratings of ongoing pain (B), hyperalgesia to mechanical (C) and cold stimuli (D) in patients with SMP. The injections were administered at t = 0 min. The time course of the vascular effects and the sensory effects of sympathetic blockade were virtually identical indicating that all of them are likely to be direct effects of the reduced sympathetic transmitter release.

occurred on the average 4 min before the increase in skin temperature. Although this difference was not significant, it indicates that temperature changes do not precede pain relief. Patients with faster temperature increase also tended to experience faster pain relief (r = 0.65). The stimulus-evoked pain followed a similar time course as the ongoing pain (Fig. 4, *right*). Due to the severity of the pain evoked by light touch (mechanical hyperalgesia) or mild cooling (cold hyperalgesia), these tests were done less frequently than the VAS ratings, and were thus averaged over bins of 10 min duration. When the test stimuli initially chosen were no longer painful during the block, more vigorous stimuli were also tried. These included bearing full body weight on a previously hyperalgesic site, deep pressure or prolonged contact with a 0°C brass probe. In no case did any of these stimuli rekindle hyperalgesia.

The time course of recovery from the block was monitored on four SMP patients (Fig. 5). In one patient, the ongoing pain returned to baseline levels within the observation period. The other three patients experienced pain relief for the rest of the day. In all 24 patients, the ongoing pain eventually returned to baseline values within the day of the block or the following day. Permanent pain relief from a single sympathetic ganglion block thus was not observed in this series of patients.

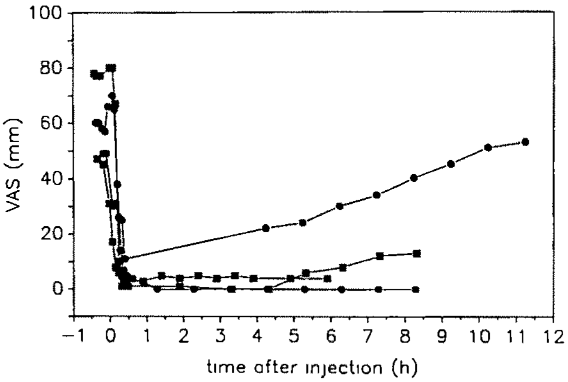

FIG. 5. Time course of recovery from sympathetic block in four SMP patients. Visual analogue scale ratings were obtained every hour, such that the patient could not see the previous ratings. Monitoring was stopped when pain had returned (n = 1) or at bedtime (n = 3). In the latter three patients, pain returned during the following day ●, cervical; ■, lumbar.

DISCUSSION

This study demonstrates that the relief of neuropathic pain and hyperalgesia by sympathetic ganglion blocks can occur within a few minutes. This indicates that the neural changes associated with SMP are highly plastic even in patients with long-standing disease. Between patients there was considerable variability in the degree of pain relief. This variability did not correlate with measures of adequacy of the sympathetic block. The relief of hyperalgesia, however, correlated with that of ongoing pain (Loh and Nathan, 1978; Wahren *et al.*, 1991). This indicates that in those patients who benefit from sympathetic block, the mechanisms of ongoing pain and of cutaneous hyperalgesia to mechanical and cold stimuli may be closely related. It also indicates that patients with SMP get pain relief even from an incomplete block, and patients with SIP get no relief even with a near maximal sympathetic blockade.

As with any procedure, a possible confounding factor is the placebo effect. Due to the invasive nature of sympathetic ganglion blocks, placebo injections were not performed. In 16 of the 24 patients, however, a second sympathetic block was performed using a new and less invasive technique that was placebo controlled (Raja *et al.*, 1991): the α-adrenergic antagonist phentolamine was given systemically through an i.v. catheter in an unaffected limb. Two placebo responders were identified in this way; they are not included in the present data. Another confounding factor is the possible systemic effect of local anaesthetics (Boas *et al.*, 1982). Tocainide, for example, is used to treat trigeminal neuralgia (Lindström and Lindblom, 1987). Its effect is possibly due to inactivation of sodium channels (Schwarz and Grigat, 1988) in the membranes of ectopic action potential generators such as the dorsal root ganglion cells. Systemic effects of local anaesthetics are unlikely in our study, because most of the patients obtained the same amount of pain relief from phentolamine (Raja *et al.*, 1991).

The inverse relationship between the initial skin temperature and the extent of temperature change (Benzon and Avram, 1986) suggests that the final temperature after sympathetic ganglion blockade is a good measure of block adequacy. Extrapolation of our regression line suggests that a skin temperature of 36°C is indicative of a complete block of vasoconstrictor tone at a room temperature of 24°C. The inverse relationship between initial temperature and amount of temperature change is consistent with the observation that temperature changes were greatest at the most distal site measured (toe or fingertip). As noted in Fig. 1, the usual proximal-distal temperature gradient (proximal warmer than distal) is reversed under the conditions of a sympathetic block. One mechanism that may account for this reversal is the opening of arterio-venous shunts in the fingers and toes that subserve a thermoregulatory function. The practical consequence of this observation is that the adequacy of a sympathetic block should be monitored with skin temperature measurements at the distal fingers or toes, independent of the location of the painful area.

Several hypotheses have been proposed to account for the pain relief induced by sympathetic ganglion blocks. Some reports in the literature indicate that the affected limb in SMP may be substantially cooler than symmetric unaffected sites (e.g. Bonica, 1979; Schwartzman and McLellan, 1987). Cooling the skin is among the stimuli that cause pain in patients with SMP (Engkvist *et al.*, 1985; Frost *et al.*, 1988; Wahren *et al.*, 1991). It may thus be that the vasodilatation caused by the block increases limb temperature and thus decreases cooling induced pain. This explanation is unlikely for the following reasons. (i) When pain was relieved by the block, even prolonged cooling with a brass probe at 0°C was not painful. (ii) Passive warming of the limb affected by SMP does not necessarily lead to pain relief. (iii) Pain relief occurred about 4 min earlier than the rise in skin temperature. Since vasodilatation preceded the change in skin temperature by 1–3 min, pain relief occurred as soon as there was evidence that sympathetic neurotransmitter release had decreased. (iv) Systemic phentolamine blocks relieved pain and hyperalgesia, but typically had little effect on skin temperature (Treede *et al.*, 1991). (v) With guanethidine blocks, pain relief often outlasts the vasodilatation by many days (Loh *et al.*, 1980) (vi) Hyperalgesia for heat stimuli, when present, could also be relieved by sympathetic blocks (Wahren *et al.*, 1991).

Thus it is likely that sympathetic neurotransmitters have a direct effect on afferent nerve endings in SMP. Sympathetic nerve endings may release a variety of transmitter substances other than norepinephrine (for review *see* Burnstock, 1986). For example, the vasoconstriction produced by sympathetic nerve stimulation has two different components in all thermoregulatory or cutaneous arteries investigated. One is mediated by the action of norepinephrine on α-adrenergic receptors (α_1 in most, but not in all, vessels). The other is mediated by a non α-adrenergic mechanism, possibly purinergic (ATP) or 'gamma-adrenergic' (for review *see* Hirst and Edwards, 1989). Nevertheless, it seems to be the action of noradrenaline on α-adrenergic receptors that is important in SMP for the following reasons. Norepinephrine rekindled pain in patients after treatment for SMP, but was not painful in normal skin (Wallin *et al.*, 1976; Davis *et al.*, 1991). Systemic α-adrenergic blockade with phentolamine was equally effective as sympathetic ganglion block in relieving pain and hyperalgesia (Arnér, 1991; Raja *et al.*, 1991). Likewise, oral phenoxybenzamine, an α-adrenergic receptor antagonist, was shown to be an effective treatment for SMP, albeit with cumbersome side-effects (Ghostine *et al.*, 1984). The receptors involved are likely to be of the alpha$_1$ type, because the α_1-antagonist prazosin and the α_2-agonist clonidine, which reduces noradrenaline release, both have beneficial effects in SMP (Abram and Lightfoot, 1981; Davis *et al.*, 1991).

As discussed in the Introduction, there is now ample evidence that mechanoreceptors are responsible for the touch-evoked pain of SMP and that a sensitization of central nociceptive neurons to this input occurs. This evidence led Roberts (1986) to suggest that excitation of mechanoreceptors by sympathetic neurotransmitters plays an important role in SMP. An influence of sympathetic efferent activity on mechanoreceptors has been documented (for review *see* Akoev, 1981), but the physiological role of this interaction is unclear and it may not occur in human skin (Dotson *et al.*, 1990). In Roberts' model, the low-grade mechanoreceptor input that may be elicited by sympathetic neurotransmitters is responsible for maintaining the central sensitized state. This model predicts that mechanical stimuli during a sympathetic block should override the block effect. While we cannot exclude that such an effect played a role in the 15 patients with SIP, the present paper clearly demonstrates that in patients with SMP, pain relief is obtained by sympathetic block despite the excitation of mechanoreceptors. Mechanoreceptor activity of the level elicited by our sensory testing thus was not capable of maintaining central sensitization. Moreover, Price *et al.* (1989) demonstrated that electrical stimulation of Aβ-fibres after sympathetic block was not painful.

We thus favour the hypothesis that activity of nociceptors is necessary to maintain central sensitization (Campbell *et al.*, 1988*a*). In SMP, ongoing nociceptor activity is probably due to an increased sensitivity of nociceptors to normal levels of sympathetic neurotransmitter release, since micro-neurographic recordings in humans failed to demonstrate sympathetic hyperactivity in SMP (Wallin *et al.*, 1976). Increased α-adrenergic sensitivity in injured nociceptors has been demonstrated experimentally (Roberts and Elardo, 1985; Sanjue and Jun, 1989; Sato and Perl, 1991). The sympathetic blocks, in this model, relieve pain because the low-grade nociceptor input to the CNS, ordinarily maintained by sympathetic discharges in the peripheral tissues, is eliminated. This elimination of nociceptor input rapidly reverses the central sensitization such that input from low-threshold receptors no longer evokes pain. Also in SIP, a similar mechanism might be involved. In this case, however, the ongoing nociceptor activity that maintains central sensitization is independent of sympathetic innervation, and may arise at a site of nerve injury in the dorsal root ganglion or elsewhere in the peripheral nervous system. Elimination of such a presumed focus of nociceptor discharges was recently shown to relieve mechanical hyperalgesia in remote skin areas (Lynch *et al.*, 1990).

Pain relief by guanethidine blocks usually occurs within 20 min (Loh *et al.*, 1980). In that procedure, circulation to the involved limb has to be temporarily interrupted by a blood pressure cuff, and the onset of pain relief may thus be due to ischaemic nerve block rather than to sympathetic blockade (Campbell *et al.*, 1988*b*). The rapid onset of pain relief in our study cannot be due to this confounding factor, and thus indicates that CNS sensitization in SMP is quickly reversible. In SMP, central sensitization thus depends on continuous input to the central neurons. This is similar to behavioural hyperalgesia (Kayser and Guilbaud, 1987) and thalamic neuron sensitization after carrageenin inflammation in rat (Guilbaud *et al.*, 1986), which could be suppressed by local anaesthesia of the injured area. In contrast, in the rat model of flexor reflex facilitation, the central sensitization due to intra-articular mustard oil injection lasted for at least 90 min beyond a local anaesthesia of the injected joint, if the local anaesthetic was given after mustard oil but not before (Woolf and Wall, 1986). Thus, in this animal model no continuous input from the injury site was necessary to maintain the central sensitized state. The long-term sensitization in that model usually disappears within 2–3 h. It is thus possible that a central sensitization that is initiated by a strong afferent barrage has to be maintained by low-grade nociceptor activity in order to persist beyond a few hours. In the present study, the initial pain returned in all patients after the effect of the sympathetic block wore off. In contrast to other studies (e.g. Arnér *et al.*, 1990), we have never observed prolonged pain relief after a single nerve block. This indicates that in many cases the underlying pathology was not removed by the blocks.

In summary, the hyperalgesia of neuropathic pain is highly plastic, even when the disease has persisted for months or years. Mechanical hyperalgesia in sympathetically maintained pain is thought to be due to central sensitization such that activation of $A\beta$-mechanoreceptors evokes pain. This sensitization of central pain signalling neurons is quickly reversible under a sympathetic block. We propose that ongoing input from peripheral nociceptive afferents is necessary to maintain central sensitization. This nociceptive input may be due to an excitatory action of sympathetic efferents on sensory nerves that is independent of neurovascular transmission and involves α-adrenergic receptors.

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BOOK REVIEWS

Neurobehavioural Sequelae of Traumatic Brain Injury. Edited by R. Li Wood. 1990. Pp. 359. London: Taylor and Francis. Price £29.95

Traumatic brain injury is the most common cause of cerebral injury during some of the most productive and formative years, 15–30 yrs. As noted in this book the best estimates of the incidence of traumatic brain injury are 6–41 per 100 000 population with a prevalence that is more than twice this incidence. Clearly traumatic injury is an important source of neurological dysfunction. The focus of the book, however, is on one aspect of this health problem, the neurobehavioural changes that follow severe traumatic brain injury. The book consists of 15 chapters which have been divided into six sections: Introduction; Identifying and describing neurobehavioural sequelae; Understanding the nature of neurobehavioural sequelae; Treatment methods 1: Psychological; Treatment methods 2: Pharmacological; Evaluating treatment outcome. The chapters are written by 20 contributors from the USA and Europe representing a variety of fields including, neurology, neurosurgery, psychiatry, psychology and rehabilitation.

The book begins with an introduction that attempts to define the neurobehavioural approach in rehabilitation of the behaviour of the traumatically brain injured. In the second chapter of this section the authors provide a thorough and balanced review of the epidemiology of the problem, providing convincing data from which they conclude that traumatic brain injury has an impressive financial impact on society and that the survivors have many unmet rehabilitation needs.

The second section describes the behavioural sequelae. The three chapters describe methods for trying to predict and identify disturbed behaviour, the types and timing of disturbed behaviour and a questionnaire to assess neurobehavioural problems. These chapters provide an excellent description of the problems of uncovering and describing behavioural problems in the traumatic brain injured individual. Chapter four of this section also describes the temporal profile of the behavioural changes, providing data showing that the behavioural problems often do not decrease in severity over time. The social impact of these problems are also described and some of the predictors of social consequences are defined. The section ends with a description of a questionnaire for the assessment of neurobehavioural problems, the European Head Injury Evaluation Chart. This chart is a questionnaire developed by a committee of experts from the European Economic Community. While this questionnaire has some excellent features no data are presented regarding the acceptance or use of the questionnaire by those dealing with head injured patients. In addition, the questionnaire lacks validation. Since it occupies 10 pages, placing it in an appendix at the end would have been more appropriate for most readers.

The third section is one of the strongest in this book. In this section a good description of the proposed pathophysiological mechanisms for the cognitive deficits and behavioural problems of traumatic brain injury are delineated. Specific post-traumatic features are defined which predict cognitive and behavioural problems. The importance of focality and extent of insults is appropriately emphasized in these chapters. A separate chapter is devoted to the role of the supervisory function of the frontal lobes. In this chapter the reader is introduced to one of the behavioural models, the Norman and Shallice model.

In the fourth section, approaches to psychological treatment of behavioural changes after traumatic brain injury are described. This section includes descriptions of conditioning procedures, of cognitive remediation, and of a therapeutic milieu for undesirable behavioural changes. Readers unfamiliar with these approaches will find them described in detail. However, the text is too lengthy to justify these varying approaches when there is simply insufficient data to favour one approach over another. The last chapter in this section, Chapter 11, is an overview of the ideal for treating these behavioural problems. To illustrate methods employed in a therapeutic milieu a case history is used. The reader is carefully led through this unfortunate young women's struggle to overcome her deficits being provided with details outlining the methods of this treatment.

The fifth section presents a highly efficient review of the pharmacological treatment of aggressive behaviour, mood disturbance and cognitive disorders. The review is comprehensive and thorough. Drug treatment protocols for treatment of aggressive behaviour are provided, and an organized evaluation of aggressive disorders to provide proper diagnosis before drug treatment precedes these protocols. This section of the book would be quite useful for many health professionals dealing with behavioural disorders in other patients as well as those with traumatic brain injury.

The final section of the book will be of interest to those in health care who must decide on methods of treatment for traumatic brain injured patients. This section describes the difficulties of studying behaviour in humans. The tables in the last chapter provide extensive information about approaches to alleviate the memory difficulties that are so often found in traumatic brain injury. The balanced presentation of the data and the conclusions that follow at the end of this final chapter are a good summary of the state of this field. Much additional research is needed while this population is being treated.

This book undertakes a Herculean task of succinctly defining the behavioural problems after traumatic brain injury, exploring the origins of these problems, describing current treatment choices and the approaches and questions for future studies. The book accomplishes this end but not without snags. It suffers from the usual disjointed style created by multiple authors. Conflicting approaches are presented with contradictory conclusions about their efficacy. Nevertheless, this disjointed approach does leave the reader with the proper conclusion that traumatic brain injury is a very important medical and social problem for which there are only early attempts at understanding and treatment. The book can be recommended as an excellent starting-point for anyone interested in the topic. The references while not extensive are properly selective, allowing any reader to pursue the topics covered in greater depth. The book can be highly recommended to health professionals in neurosurgery, neurology, occupational therapy, rehabilitation, speech therapy and nursing.

ROBERT S. GREENWOOD

Amyotrophic Lateral Sclerosis and Other Motor Neurone Diseases. *Advances in Neurology*, Volume 56. Edited by Lewis P. Rowland. 1991. Pp. 569 New York: Raven Press. Price $169.00.

In 1981 a major symposium on Amyotrophic Lateral Sclerosis (ALS) and related diseases, sponsored by the USA Muscular Dystrophy Association (MDA), was held in the USA. This was highly successful and its numerous presentations provided the basis for the subsequent publication of an important volume in the *Advances in Neurology* series, namely *Human Motor Neuron Diseases* edited by Lewis P. Rowland which appeared in 1982. This book was wide-ranging in its scope, describing basic aspects of the neuronal growth and function as well as virtually all known aspects of the clinical, epidemiological and pathogenic disease spectrum. The book was quickly established as an essential reference work in this difficult field and more than a decade after its publication it is still regarded by many as a rich source of ideas and data. In view of the various advances in the field which had been made since the appearance of the first volume, an *ad hoc* Program Committee of the MDA decided to organize a further major conference on ALS and this was held in Arizona in 1989. There was also a clear need to produce a new book on the subject which would not only provide an update of several of the themes which had previously been covered, but also reflect the changes in research emphasis which had occurred over the previous 10 yrs. For this purpose it was decided not to provide a new edition of the 1982 book but instead to publish a sequel emanating from the 1989 ALS Conference. In this endeavour the editor of the new volume, again Lewis P. Rowland, was faced with two formidable challenges. First, it was essential for the new book to maintain the exceptionally high standard of its predecessor regarding both quality and depth. Secondly, there arose the obvious difficulty of organizing the various aspects of the subject in such a way as to provide both a coherent and logical presentation of diverse aspects of ALS as well as cover the basic neurobiological aspects of motor neurons and their growth. The editor of *ALS and Other Motor Neuron Diseases* succeeded on both counts, for the wide range of talent (114 mainly North American contributors) that he has assembled has coalesced to produce a book which is not only highly informative and comprehensive but also stimulating and, on the whole, enjoyable to read.

The introductory chapter to a multi-authored book of this kind is of critical importance in both providing a balanced and informative overview of the subject and in explaining the rationale and organization of the chapters written by the numerous authors. By any standards Rowland's introduction is outstanding, and he discusses 10 central themes in ALS research over the preceding decade. The themes include clinical problems, basic neuroscience, epidemiology, pathology and pathogenesis and they aptly reflect the diversity of the problems and the heterogeneity of the ALS syndromes. For instance, some readers would probably be surprised to read that there are no less than 16 different syndromes of ALS and dementia, 28 autosomal dominant motor neuron disorders, 37 autosomal recessive forms and 7 X-linked forms. No doubt these numbers will increase well before the next major volume on ALS is produced.

We are presented with no less than 46 chapters which, excluding the introduction, cover the general fields of Cell Biology (6 chapters), Cellular Pathology (4 chapters), Genetics (9 chapters), Epidemiology and Environmental Factors (9 chapters), Immunology (11 chapters), Persistent Viral Infections (4 chapters) and Clinical Trials (2 chapters). The general standard is high although, as would be expected in such an endeavour, there is some noticeable variation in the length and quality of the individual chapters. Regarding the former, the length varies from 4 pages to over 20 pages. As regards relevance, the choice of all the chapter subjects is justified in relation to ALS although the importance of certain topics is more immediately apparent than others. The organization of the chapters is logical although I thought that the three cogent contributions discussing the separation of motor neuron diseases from pure motor neuropathies might well have been more appropriately included in an early clinical-pathological section rather than being subsumed under the immunology rubric. The chapters would also have benefited from rather more unity of style; for example, summaries are not always provided and in some cases clear divisions into important subheadings are not used.

The section on Cell Biology is concerned with relevant aspects of neuronal biology, including *in vitro* analysis of motor neuron lineage using a retroviral method, *in vitro* studies of cultured motor neurons including their dependence on various trophic factors and reviews of spinal motor neuronal morphology and neurofibrillary proliferation. It certainly seems prudent for motor neuron disease funding bodies to continue to support such basic neurological research since vital clues to the causation of the human disease may be gleaned from them. Likewise, studies of the cellular pathology of motor neuron disease are important, partly because the apparently restricted pathology of the disease may well reflect disease causation, and partly for the insight that such studies have recently given into disease pathogenesis. The latter is aptly reflected by the chapter from Leigh and Swash on Cytoskeletal pathology including the important finding that spinal cord neurons from motor neuron disease patients contain ubiquitin inclusions. The detailed biochemical analysis of ubquitinated proteins offers a real challenge for the future.

The genetics section is of particular interest and importance since the application of molecular genetic techniques to muscle disease, notably the identification of the gene which causes Duchenne Muscular Dystrophy, has been highly successful over the past decade. Whether these techniques will identify the gene or genes responsible for certain forms of familial ALS is as yet unknown, but genetic analysis of the spinal muscular atrophies has already proved successful. Thus, as Rowland states in the preface, the gene for spinal muscular atrophy was localized to chromosome 5 and the allelic nature of the infantile and juvenile forms of the disease was demonstrated after the 1989 meeting had taken place. Moreover, gene linkage studies of familial ALS are already underway and the pace of this type of research is such that one is left in little doubt after reading this section that the molecular analysis of ALS will prove to be of great interest and relevance

There has been abundant interest for some time in epidemiological and toxic environmental aspects of ALS, and these are well covered in this section of the volume. Prominence is rightly given to epidemiological aspects of Western Pacific ALS and the possible role of the cycad neurotoxin β-methyl-amino-L-alanine in producing the ALS-Parkinsonism Dementia complex in this region. There are also interesting chapters on the possible role of non-N-methyl-D-aspartate receptor mediated neurotoxicity and altered glutamatergic mechanisms in producing neuronal damage in the disease. Useful reviews of aluminium-induced motor neuron degeneration and post-radiation motor neuron syndromes are also included.

Immunological studies of ALS have generally been disappointing and until recently hard evidence for a primary role of immune-mediated neuronal cell destruction has been lacking. However, recent observations have allowed fresh evaluation of this whole area and these are very well covered in this section. The latter is wide-ranging and is concerned with immunological studies in both ALS and motor neuropathies. Of particular importance was the demonstration of anti-GM1 ganglioside antibodies in a proportion of patients with ALS as well as other causes of lower motor neuron syndromes such as motor neuropathy. These findings provide a rational basis for immunosuppressive treatment in these various patient subgroups and indicate exciting new therapeutic possibilities in ALS. Animal models such as experimental autoimmune motor neuron disease are also providing fascinating insights into the possible role of immunopathogenic mechanisms in motor neuron disease and these are well described by Appel *et al.*

In view of the association of motor neuron disease with a previous episode of paralytic poliomyelitis, and the affinity of poliovirus for anterior horn cells in the spinal cord, the possibility that motor neuron disease may be caused by a virus has been entertained for many years. It was therefore not surprising that four chapters are devoted to the possible role of persistent virus infection in the disease. The enigma of the post-polio syndrome is described very well by Dalakas and Illa and I concur with their view that this is most likely to be the result of attrition and premature aging of anterior horn neurons rather than persistent poliovirus infection. Animal models of retrovirally induced anterior horn cell damage are very well described in this section and are clearly fascinating. While they certainly merit intensive study it is unclear just how much relevance the insights gained from such studies will have in relation to the human disease.

The editor comments in the Introduction that despite the considerable recent advances in basic neurosciences, there has been little application of this understanding to clinical problems such as ALS. The lack of therapeutic advances for ALS patients is aptly reflected by the two lone chapters on clinical trials in the disease. The detailed account of clinical trial design in ALS by Brooks *et al.* is particularly clear and the importance of including careful muscle strength measurements is emphasized. Overall, I was left with the strong feeling that the really important advances in ALS are most likely to come from molecular genetic, immunological and perhaps toxicological studies, but our relative ignorance of the aetiology of this dreadful disease makes meaningful speculation particularly difficult.

The book is extremely well produced with superb illustrations and diagrams, has a good index and is generally well referenced. Unfortunately, it is expensive by any standards and is likely to be out of reach of the pockets of most doctors in training. Nevertheless, it is an excellent volume which represents the 'state of the art' on the subject of motor neuron disease and related diseases. As such it will serve as an authoritative reference source and should be on the shelves of all neurological libraries. All neurologists and many other physicians and clinical scientists concerned with the investigation and management of patients afflicted with these enigmatic diseases will benefit considerably from owning this book and I am delighted to have a copy.

PETER G. E. KENNEDY

Culturing Nerve Cells. Edited by Gary Banker and Kimberly Goslin. 1991. Pp. 453. London: The MIT Press. Price £44.95.

If you have not gazed upon a dish of neurons which have been busily growing their delicate processes and seeking contact with desirable companions, then I suggest that you go to a colleague's laboratory and do so. The delight of observing cells cultured from the nervous system comes not just from the fact that there they are, available for whatever experiment you have in mind, but they are also quite beautiful. There is also the possibility of an added appeal that the growth of 'bits of brain' in an incubator, like genetic engineering, may have for the hidden Baron Frankenstein. But the aesthetic and subconscious satisfaction apart, it is true that cell culture has proven to be a powerful methodology for the experimental study of cell development and behaviour. First seriously applied to studying the nervous system some 40 to 50 yrs ago it has in the last 10 yrs become a technique of major importance in neuroscience. In the *Journal of Neuroscience* 15 papers out of the 60 published in October and November of 1991 used cell culture as the major or as a significant part of the experimental procedure. The predominance assumed by tissue culture has probably been inevitable given the variety of cells, particularly within the central nervous system, and the complexity of their interactions. Tissue culture alone has been responsible for changing our awareness of the importance of the locally produced active peptides and proteins with cytokine and growth factor properties in the development and maintenance of the nervous system. Additionally, the general increase in the use of tissue culture for both research and commercial reasons has itself led to the availability of high quality plastics and chemicals at a reasonably low cost which in turn has made the whole procedure more reliable and successful.

There are a number of books and manuals dealing with the tissue culture of the nervous system already available, so does this volume provide anything new? It is the first in a series intended to deal with cellular and molecular neuroscience to be produced by MIT Press, and is intended to be a 'do-it-yourself' manual.

It is a weighty, well-designed hardback book, not the familiar spiral-bound soft-back practical manual which is usually the product of an advanced training course. This, however, is a book, not just a manual, so large parts of it can be read for general instruction. But do not be deceived by appearances, it contains a wealth of practical detail in each of the chapters, written by authors experienced in the use of the procedures described. Most importantly they also outline the reasons why they personally employ tissue culture to study the problems which interest them. All stages of the protocols are covered in detail, but the basic procedure is frequently summarized and sometimes a timetable is also included. It is true that you learn more about a technique when things go wrong but it can be frustrating. The authors all provide troubleshooting advice and although it will not cover all possibilities these workers have a good idea of what are the commonest problems to arise.

The illustrations, line-drawings and half-tones are of good quality, although some of the photographs provided to help in the dissection of foetal tissue are rather murky. Probably nothing can quite replace a live demonstration.

Apart from the initial four chapters which deal with the generalities of culture and procedures of general applicability such as immunostaining and electron microscopy which are so important for characterizing the cells in culture, eight of the 15 chapters are concerned with the culture of specific groups of neurons. Some of these deal with the particular problems of establishing low density cultures in which the growth of the neuronal processes can be seen more clearly. There is an excellent chapter dealing with the establishment of cultures to study myelination by either oligodendrocytes or Schwann cells. Glia, recognized in the introduction as presenting difficulties, are recognized by a single chapter on the culture of astrocytes. This is perhaps the major deficiency of the book.

The book's physical presence seems to imply that this is not just meant to be a 'state-of-the-art' update, but is a definitive work. It is quite possible that it is for the systems given, and within the limitations imposed by the interests of the authors. But no methodology is static, new materials become available, ancillary equipment is developed and objectives change, so I am quite sure further practical manuals of neural tissue culture will appear. To take one example, virtually all of the workers writing in this book have as part of their interest the development of the nervous system, and more specifically the role of cellular interactions in such development. This may be an inescapable outcome from the fact that the cells from developing tissues are relatively easy to grow and with the exception of neurons from the invertebrate Aplysia and the use of the neuroblastoma cell line PC12, all of the systems described are based on the use of foetal or early post-natal animals as the source of cells or tissues. However, there is an increasing interest in the processes of repair and degeneration in the mature central nervous system and because of this there is a demand for methods for culturing cells from adult mammalian tissue. Papers on the culture of adult retina, retinal ganglion cells and sensory neurons have indeed already been published.

There is still some reticence about accepting results obtained using tissue culture as completely physiologically relevant, a view recognized and countered by Bray in his chapter on the use of isolated chick neurons for studying axonal growth. As he points out, if the process of interest appears to proceed in a manner quantitatively not dissimilar to that in the animal, then it is unlikely that this would be being produced by a dissimilar mechanism. Thus the advantage of the culture dish is that it provides a means of making dynamic observations under controlled conditions which would be impossible in the intact animal. The behaviour of the growth cone is a fairly well-defined problem but I am not sure that the same argument can be applied so readily to all studies. There is a danger that the culture system can be too severe a reduction. The observation that a cell requires, or responds to, a particular growth factor to provide a change comparable to that seen *in vitro* may be quite correct, but danger lies in extrapolating this to a point where the complexity of the physiological system is ignored or conveniently forgotten. Possibly the most difficult facet of the physiological state to imitate or compensate for in the culture dish is the dynamic state of the milieu, something particularly true in development. Since there are so many recognizably distinct types of neuron one very important question in developmental neurobiology is to what extent is the phenotype of these cells determined by epigenetic factors and to what extent is it intrinsic, determined by lineage? The answer is very important in terms of the particular neuron to be further studied in culture. Has the cell *in vitro* been derived from an animal at such an age when the cell's character has already been determined, or is there sufficient remaining plasticity such that its final properties *in vitro* may be determined by what other cells have by chance survived and their proportions in the dish? Thus in the chapter on the culture of mammalian autonomic neurons it is pointed out that co-culture of these with non-neuronal cells will influence the morphological and biochemical differentiation of the neuronal cells. Furthermore, the medium used can strongly influence the proliferation and trophic factor secretion of the non-neuronal cells. The chapter on the culture of neocortex and basal forebrain by Baughman *et al.* indicates how fully these questions can be answered using a combination of pharmacological, physiological and immunological techniques to define the cells, their proportions and connectivity The question of phenotype is also of importance in cell lines, such as the popular pheochromocytoma cell line PC12. Here it is pointed out that proliferating cells have an increased liability to mutate and that subclones can be selected unwittingly by using different culture conditions and a plea is made for the use of standardized culture conditions and the evaluation of the cell's phenotype to make sure that it conforms to the original line as closely as possible.

The final chapter deals with the organotypic-slice culture as applied to the nervous system. This type of culture was the earliest to be described, by Ross Granville Harrison in 1907. The first figure in the book is one of Harrison's of nerve fibres, complete with growth cones, growing from explanted embryonic frog spinal cord first published in Anatomical Records in 1910. Slice cultures are maintained in roller tubes and are not easy to establish, seeming rather more susceptible to inconsistencies in technique or materials. Patience and persistence are considered to be essential if one wishes to embark on this type of culture. Although slices undergo significant morphological changes and thinning they remove some of the objections that are inherent to those systems starting with cells from dissociated tissue, for as nearly as possible all of the cells originally present in the piece of tissue of interest are preserved and to start with are there in 'physiological' proportions. Changes occur during the isolated tissues' adaptation to the *in vitro* status, but certainly in, for example, the cerebellum and the hippocampus, cellular relationships are well preserved. Generally the neural cultures which are used are fine for studying the effects of the factors in the local microenvironment on development but are not so good for examining the mutual interactions between more remote regions of the brain. However, the slice culture using co-cultures of different regions is ideally suited for this purpose.

This book should prove a most useful technical reference book for those who wish to start in neural tissue culture. But also since there is a tendency for the culture laboratory to specialize in certain systems, as exemplified by the contributors to this volume, even those already using the technique should find it useful if they wish to establish unfamiliar or new systems and perhaps in updating some aspects of their own methods.

N. A. GREGSON

The Neurology of Eye Movements. Second edition. By R. J. Leigh and D. S. Zee. 1991. Pp. 561. Philadelphia: F. A. Davis.

In 1983 the publication of *The Neurology of Eye Movements* by John Leigh and David Zee provided the definitive tome on this fascinating area of clinical neurology. Now, some 8 years later, the second edition has appeared bigger and better than its predecessor.

Despite all the advances in todays molecular neurobiology much can still be learnt about brain function by the analysis of motor function. Moreover, continued interest in the neurology of eye movements provides the clinician with a wealth of disturbances which can be increasingly correlated with the neuroanatomy and neurophysiology of a variety of neural pathways. Indeed it is some 35 yrs since the publication of the previous definitive clinical book, the *Neurology of the Ocular Muscles* by David Cogan, in which these early correlations were first so clearly laid out. With an understanding of the latest ocular motor neurophysiology provided in Leigh and Zee's new edition the clinical neurologist can return to his/her roots: bedside diagnosis and localization of a disease process, and for a moment relax from the rigors of such topics as Western blots and mitochondrial respiratory chains.

In the 8 yrs between the two editions there has been a dramatic increase in our knowledge of the basic anatomy and physiology of eye-movement control, and their disordered control as seen in patients. Indeed the number of cited clinical papers has more than doubled during this period. Why then has interest in eye movements continued at such a level despite the greatly increasing amount of research into motor control of limb and axial movement? To put it another way, what can eye movements teach us about movement in general? This was addressed a few years ago by David Robinson in an essay entitled, 'Is the ocular motor system a cartoon of motor control?' Undoubtedly, the major advantage of studying eye movements in comparison with axial or limb musculature is their relative simplicity. Eye muscles lack a classical monosynaptic stretch reflex, and they move a constant load. Pairs of muscles are reciprocally innervated and the control system does not have to deal with the problem of multiple joints which are involved in limb movements. The fact that all the neural machinery for eye movments is contained within the cranium has enabled neurophysiologists to record the single unit activity at all levels permitting a bottom-up approach. The somatomotor physiologists on the other hand have to use a top-down approach beginning with premotor structures, because of their difficulty in recording from neurons in the spinal cord of behaving mammals. However, perhaps the two major factors which have led to such interest in eye-movement control are firstly the relative ease and precision with which eye movements can be recorded, and secondly the observation, dating from Dodge early this century, that several different classes of eye movements can be distinguished, each with a specific function. Subsequently each class was found to have a distinct anatomico-physiological substrate.

In this book a separate chapter is dedicated to each of these classes, saccades, smooth pursuit and fixation, vestibular-optokinetic and vergence, in which their normal function, anatomical substrate, physiology and clinical disturbances are fully described. The clinical neurologist will also welcome the sections on the clinical examination of each class of eye movement. This is often inadequately performed at the bedside so diminishing the clinical returns from the examination. Regrettably talk of gains, pulse-steps and velocity storage by 'professional' oculomotor experts tends to cause other neurologists to switch off rapidly. This prevents them from appreciating that a little refinement in their normal clinical eye-movement examination technique will greatly enhance their ability to detect abnormalities. Quantitative evaluation of eye movements in the laboratory is not a pre-requisite for determining many abnormalities useful in clinical diagnosis.

This second edition is remarkably up to date with most of the literature up to and including 1990 cited. As a reference source it is unbeatable and every neurological subspeciality requires such a monograph both to enable new recruits rapidly to find their way about the literature, as well as enabling references relevant to an interesting or unusual case to be accessed easily.

There is only one completely new chapter in this edition resulting from the increasing interest in the mechanism by which gaze is held in an eccentric position. The ocular motor neurons carry a neural signal that contains velocity and position components, the latter being derived by a process of mathematical integration from the velocity-coded signal.

This process, necessary for all versional eye-movement commands, has recently been found to be carried out in a neural network, called the neural integrator, located in the medial vestibular nucleus and nucleus prepositus hypoglossi, and the nucleus of Cajal, for horizontal and vertical eye movements, respectively. This chapter provides a very clear account of these mechanisms for gaze holding and their abnormalities such as gaze-evoked mystagmus.

Since the first edition considerable advances have also been made in relation to the cerebral control of saccadic eye movements and the neural pathways for smooth pursuit. These advances have been achieved by neurophysiologists recording from alert nonhuman primates, and by clinicians studying patients with focal lesions of either structural or neurotransmitter type, and are fully and critically described. The last two chapters deal with the diagnosis of peripheral ocular motor palsies and strabismus, and central disorders of ocular motility and will therefore be of particular value to the clinical neurologist.

Because of the frequency with which disturbances of eye movements are found in clinical practice, a copy of *The Neurology of Eye Movements* should be in every departmental library. The first edition became the definitive account and this second edition has only served to enhance its position as one of the outstanding monographs in clinical neurology. Those neurologists who have often been fascinated by the rich variety and sometimes bizarre nature of eye movements should buy a copy so that their eye-movement discoveries will be even more rewarding.

Finally, the basic scientist, neurophysiologist, neuroanatomist or bioengineer with an interest in motor control will find this volume a useful account of ocular motor control. This 'simple' motor system is rapidly yielding to the combined efforts of basic and clinical scientists and this book provides a first-rate 'state of the art' account.

CHRISTOPHER KENNARD

Visual Agnosia. By M. J. Farah. 1990. Pp. 202. Chichester: John Wiley and Son. Price £22.50.

Visual Agnosia provides a wide-ranging and provocative theoretical review of clinical disorders of object and face recognition. In her monograph, Martha Farah makes use of current cognitive and computational theories of normal object recognition in order to interpret disordered processing. She draws on the distinction between 'where' and 'what' to make a major subdivision between dorsal simultanagnosia and ventral agnosic failures of object recognition. The label of dorsal simultanagnosia is used to encompass visual disorientation, optic ataxia and Balint's syndrome, all of which are viewed as failures of 'attention' and are speculatively related to neglect. Ventral object agnosias, by contrast, are attributed to deficits in representing object structure. These attentional and representational subcomponents of information processing are required for the genesis of a 'grouped array' adequate for action and interaction with the environment.

Farah suggests that ventral agnosic patients either have a problem in representing the component parts of a visual stimulus (prosopagnosia with or without object agnosia) or in bringing those parts together so that they form a coherent whole (object agnosia with alexia). She proposes 'there can never be a case of object agnosia without either prosopagnosia or alexia. Second that there can never be a case of prosopagnosia and alexia that does not also have some degree of object agnosia' (p. 128). Ninety-nine cases are culled from the contemporary literature to test the hypothesis. There are two apparent exceptions but their clinical case descriptions are considered ambiguous (p. 132).

The adequacy of individual cases as a 'test' for Farah's position rests on definitional and diagnostic criteria, none of which are uncontroversial or even theory-independent. Alexia, for example, is both complex and a significant source of clinical and theoretical debate in its own right. There are also considerable problems in discriminating partial cortical blindness, the various forms of agnosia and optic aphasia from each other. No single criterion test or test battery will identify a patient's disorder, and the delineation of deficit type requires complex profile analysis including considerable weight being placed on 'null' results. The implementation and testing of Farah's theory may not be quite as straightforward as a cursory reading might suggest.

Visual Agnosia is a broad programmatic outline with particular strengths at the metatheoretical level. The overall scope of the monograph is very impressive, encompassing a multilingual literature. Farah's presentation is clear but there are (inevitably) occasional inaccuracies. There is also little or no consideration of group studies as a guide to localization or to cognitive fractionation. These disadvantages are balanced by the positive value of the book. It provides a focused attempt at bridge building between neurology on the one hand and cognitive science on the other.

The broad brush approach adopted by Farah produces an easy read. The book provides an excellent introduction to cognitive neuroscientific theorizing and *en passant* provides a helpful beginners guide to computational models of distributed processing. At the same time, the monograph is likely to inform and to provoke debate amongst an audience with greater expertise. Indeed, it has already become a frequent citation on cognitive psychology. The style of presentation is clear and logical and the bibliography is outstanding. It should prove both interesting and accessible to a wide and multi-disciplinary readership.

ROSALEEN A. MCCARTHY

Neurotransmitters and Epilepsy. Edited by R. S. Fisher and J. T. Coyle. 1991. Pp. 260. New York: John Wiley. Price £79.00.

The great explosion of biochemical knowledge has been the major contribution to the study of epilepsy in the past two decades. At the manuscript stage of this book, 69 neurotransmitters and neuromodulators had been identified; and since then others have been described. The field moves with bewildering speed, but all too often the neurochemical exposition is, to the uninitiated, caballistic in its complexity and meaning. A central problem for clinicians and scientists is to try to maintain bearings, to try not to get lost in a subject which is developed by small, disparate and fragmentary research studies. It is exactly in its ability to place research findings in context, that this book is so successful.

There are many pitfalls; there is, for instance, the danger of the circular heresy; the fact that one substance is intensively studied and found implicated in epilepsy does not mean that other substances, not investigated, are not more important. The large number of chemical changes uncovered in epileptic brain pose further difficulties; it is perhaps not over-cynical to note that abnormalities have been found with almost all transmitters investigated; but how many are actually relevant? Chemical mechanisms do not only result in epilepsy, but so too does epilepsy alter neurochemistry. The perennial question of chicken or egg, or cause or effect, is nowhere more pressing than in this neurochemical field. Equally, and again all too often, many chemical changes noted in epilepsy are simply epiphenoma, or secondary to the primary epileptic process, or simply a reflection of seizure spread.

The thrust of research often depends most on the availability of tools and experimental models, rather than logical developmental thought. An individual research project can answer only those questions which it addresses; a study of glutamate, for instance, will of necessity emphasize excitation, and GABA inhibition. The results of animal experimental studies too should be interpreted with caution, as there is no animal model which is an entirely satisfactory mimic of human epilepsy, and the model used may itself cause brain damage (a good example is the frequently used Kainate model of limbic status).

It is difficult for the neurochemist, with currently available tools and experimental models, to untangle these complex webs, and the extrapolation of these experimental findings to man must therefore be circumspect. One outstanding merit of this book is its success in sifting the available evidence to present a balanced and cautious overall picture. The chapters on GABA and glutamate, both important players in contemporary epilepsy are good examples; in each chapter, studies presenting evidence for and against a series of hypotheses are given clearly, the evidence is assessed in exemplary fashion, and the authors give an excellent overview.

The book has 17 chapters, each dealing with different neurotransmitters, receptor systems or investigatory methods. The contributors are distinguished in their field, and the overall standard is very high. Part of this success is due to the seemingly strict control which the editors have exerted; the book has a pleasing uniformity in style, the chapters are tightly organized, and there is very little overlap. All is underpinned with a remarkably comprehensive coverage of the very latest literature. The text is derived from a summer lecture series for neurosurgeons given at Woods Hole; its stated goal is to make accessible to the clinician the current state of biochemical play. In this intention, it is wholly successful, and it outclasses any other recent book in its field.

S. D. SHORVON

Clinical Neuro-Urology. Second Edition. Edited by Robert J. Krane and Mike B. Siroky. 1991. Pp. 692. Boston: Little, Brown and Company. Price £65.00.

Neuro-urology is the branch of urology which deals with disorders of micturition secondary to neurological disease. It is a remarkable fact that neurologists have paid so little attention to this area of medicine that the subject has been left almost exclusively in the hands of the urologists. It is almost as if neurologists have taken the view that they would not interest themselves in the function of the legs since this was more correctly the province of the orthopaedic surgeons; clearly a preposterous point of view. Why then has there been so little neurological input into the study of the bladder?

It is perhaps worth taking a little time to examine the problem. The function of the bladder is to store and subsequently to eliminate urine, and disorders of these functions result in a limited range of symptoms (chiefly either incontinence or retention), despite a very wide range of possible causative pathologies. This poverty of symptomatology, combined with the fact that there are few relevant neurological signs to be elicited, may have something to do with the lack of a significant neurological perspective. Moreover, those investigations which can shed light on the relevant pathophysiology such as urodynamic studies are usually carried out and interpreted by urologists. All this has been combined with an uneasy feeling inculcated during early medical training that some silent, potentially lethal disorder such as upper tract damage and renal failure may intervene unless the patient is properly managed by a urologist. Thus, the origins of this state of affairs are understandable. The time has now come for a change.

Continence is a severe test of neurological integrity. Normal bladder control requires intact connections between the medial parts of the frontal lobes and the most caudal parts of the spinal cord as well as normal motor cortex and extrapyramidal function. Thus the variety of possible neurological causes of bladder dysfunction are extremely interesting and varied. The basic neuroscientists are well ahead of the clinicians and have in recent years been making exciting discoveries relating to smooth muscle function, the central connections of the sacral neurons and the complexity of neurotransmitters in control of the urogenital tract. These facts have now begun to emerge at a time when there is a new willingness on the part of the patients and their doctors to discuss personal and intimate problems: possibly an unexpected and rare benefit to result from the pandemic of HIV infection.

Clinical Neuro-Urology is currently the leading authority on neurogenic bladder disorders. The first edition of this book appeared in 1979 and was seen then as the successor to the few classical texts ever written in this field, such as *Neurological Urology* by Bors and Comarr and Boyarsky's *The Neurogenic Bladder*. The second edition is certainly a timely update but as with the first edition, is written mostly by urologists for urologists.

The text is divided into five sections: basic science, clinical investigation, neurogenic vesicourethral dysfunction, non-neurogenic dysfunction, nonsurgical treatment and surgical treatment. The chapters on basic science deal with fundamental aspects of neuroscience and the chapter on the 'physiology of micturition and continence' deserves perhaps special recommendation for its brave attempt to review and synthesize the many different theories that have been proposed on this topic: covering 57 pages, it cites more than 300 references. The chapters on the fluid mechanics of micturition, detrusor muscle mechanics and clinical significance of hydrodynamics, although probably mandatory in such a textbook, have a heavy content of physics and are unlikely to be easily read by clinicians. The sections on clinical investigations are best when discussing the various aspects of urodynamics rather than neurophysiology, the latter give almost no references later than 1986 The sections on clinical disorders are extremely variable in quality: the chapter on myelodysplasia and sacral dysraphism being of particular value, whereas that on intracranial lesions affecting bladder function, somewhat urological in approach. Nonsurgical managements of bladder problems should be a particular interest to neurologists since it is by these means that their patients with progressive neurological disease will be managed. The sections on surgical management are presumably excellent since they are written by leading American urological surgeons, although surely the chapter on neurostimulation which fails to give even a reference to Brindley's work has made a serious omission?

In summary, this book is an excellent volume for the urologist interested in bladder dysfunction due to neurological disease. It will, however, come as a disappointment to neurologists seeking a neurological approach to the subject. However, they have only themselves to blame.

CLARE J. FOWLER

Paediatric Neurology. Second Edition. Edited by Edward M. Brett. 1991. Pp. 895. Edinburgh: Churchill Livingstone. Price £90.00.

One of the defining characteristics of childhood is that it is the period during which the human central nervous system grows to maturity. It follows that paediatric neurology is a unique and special study; that of the disorders of this still developing system. In reviewing the first edition of Dr Brett's book I began 'Oh, what a lovely book!' Can that still be said of the new edition? The passage of 8 yrs has inevitably brought the beginnings of corpulence (a 46% increase in the number of pages) and a fading of the dermal texture (the paper is thinner and less glossy) but the essential character of the book remains the same. The print is smaller giving about 50% more words per page so overall there must be at least twice as much text as in the first edition. An unfortunate consequence of the change in paper quality seems to be some of the illustrations, especially the CT scans are darker with loss of contrast and clarity.

The book has been completely revised and updated. Out-of-date references have been pruned and almost every section has been extensively rewritten and added to. In particular, therapeutic advice is more detailed and helpful. The first edition had 10 contributors of whom five remain and there are 10 new ones. There are two completely new chapters, one on acute encephalopathy and the other on neuroimaging, the latter devoted largely to CT scans. Five chapters have been written afresh by new authors. Nevertheless, the whole book bears the stamp of Dr Brett. He has written 17 of the 27 chapters, 13 of them as sole author. It is paediatrician's book for paediatricians. His delight in children is clear. Those whose dignity will not allow them to scramble around the floor playing games or to be struck with a patella hammer or even kicked for fun will not be attracted to paediatric neurology by reading this book. The conventional heel-to-

toe walking to bring out ataxia here becomes a fantastic game with the child attempting to walk a tightrope across a crocodile infested river! Eliciting the knee-jerk can be made a delight to a young child if the doctor 'places his own leg in such a position that the patient's foot will inevitably kick it as the knee jerk is elicited'. Silly? Maudlin? The cynic might say so, but I prefer to think of it as a manifestation of that enthusiasm for children which should motivate all paediatricians.

This is a book of a mature physician who has written down only the facts of paediatric neurology but the interpretation of those facts when passed through the sieve of his own immense experience, and beyond the facts, the diseases and the techniques, he shares with readers the thoughts and the feeling of an erudite and civilized man. He quotes a poem by Thom Gunn on foetal development, Queen Victoria on her dislike of young babies, Ecclesiastes on the importance of being gentle, and Alfred Stilte on the physician's aim 'to rid his patient's path of thorns if he cannot make it bloom with roses'. A footnote to the section on Sydenham's chorea gives a brief account of the life of St Vitus.

The science and the philosophy But the science is no less hard-headed for it. It is as complete an account of paediatric neurology as you will find in a single volume. Each chapter is followed by an extensive list of references and recent advances such as the discovery of dystrophin and the elucidation of the mitochondrial diseases and the peroxisomal disorders are included. The index, which was a weak point in the first edition is now much more extensive and serviceable.

No single writer can encompass the whole of the subject and they are wise who not only know their limits but to know who to approach to supply what they cannot. Dr Brett's colleagues have served him and their readers well. New chapters, by Dr Gaynor Cole on acute encephalopathy and Professor Michael Levin on infections of the nervous system, add to the value of the book as a guide to practise which paediatricians will turn to when faced with difficult clinical problems.

A mischievous carp and a touch of acral traction; the patella in patella hammer is a noun. Patella hammer is a surprising finding in such a book as this; presumably it is the neurological equivalent of a murine trap or a beery bottle.

'Oh, what a lovely book!' still? Yes, indeed. If you are a paediatrician, buy it; if you are a neurologist have it available to refer to (if only to emphasize the fact that the neurology of adults and that of children are two very different things); if you are a paediatric neurologist you will need no advice, you will already possess a copy of the first edition and be about to update it; if you are a junior doctor wondering whether paediatric neurology could be for you borrow it, immerse yourself in its flavour, and if you like the taste, take it from there. This country needs paedriatric neurologists.

D. P. Addy

Neurobehavioural Problems in Epilepsy. Advances in Neurology Volume 55. Edited by D. B. Smith, D. M. Treiman and M. R. Trimble. 1991. Pp. 507. New York: Raven Press. Price $177.50.

The Psychoses of Epilepsy. By M. R. Trimble. 1991. Pp. 224. New York: Raven Press. Price $86.

There is an unwritten assumption held by neurologists (amongst others) that psychiatric disorders are caused largely by organic dysfunctions of the brain. The proposition is seemingly so obvious that to many it requires no further thought, and where organic causes are not apparent, it simply reflects the incomplete nature of our current knowledge. Psychosis is a case in point; a search for the causes of schizophrenic 'illnesses' includes viruses, genetic abnormalities, enlargements of the cerebral ventricles, abnormalities of every conceivable neurochemical, and it has been attributed at various times to dysfunction of almost every region of the brain (the frontal lobes, the temporal lobes, the corpus callosum, the left hemisphere, the limbic system, the basal ganglia). In spite of this, would it be too strong a claim to say that we are no further advanced in our understanding of the disease than was R. D. Laing when he pronounced schizophrenics to be the only sane members of our society? One problem for all theoreticians is where to draw a line. Where does normality end and disease begin, where does human eccentricity transcend the bounds of health and where does individuality (or even its extreme expression, genius) become pathological? To the neurologist, who can take cover under an abnormal CT scan, these issues are seldom necessary to address; to the hapless psychiatrist with a disturbed patient but normal CT (and even MRI!)

the argument will not go away. I should say here that, trained as I am in the organic tradition, I take a physical view of mental illness, but I recognize the paucity of supportive evidence.

The study of the psychiatric disturbances in objective organic disease is an attractive proposition for those neurobehaviourists who seek the organic basis of mind, and epilepsy is an obvious target. Epilepsy, the most venerable of diseases, with a written history dating from ancient Babylon, has been long associated with disorders of mind, and the notion that through epilepsy may be revealed the organic basis of pathological behaviour is a powerful one; indeed, it is these disturbances of mind which make epilepsy so fascinating a condition. It could indeed be argued that it is *only* through a study of physical disease that the organic basis of psychiatry can ever be elucidated. There are (needless to say) traps for the unwary. Epilepsy is often a symptom of underlying brain disturbance, much in the same way as may be the mental changes. Thus, whilst both the fits and mental disturbance may be manifest together, they may bear no causal relationship. Correlative studies in such situations seldom yield useful information, yet are the scourge of the psychiatric literature. Loose correlation easily slips into false classification, and psychiatry is doubly burdened by an excess of new syndromes and classification schemes. Furthermore, the epileptic person may be affected by the insecurity of unpredictable seizures, burdened by the failure of ego development due to parental overprotection, influenced by the need for shelter from public embarrassment or humiliation; all such impulses may affect behaviour in a way which has nothing to do with anatomy, physiology or biochemistry nor indeed with ventricular size. The behavioural 'disturbance' in these cases is not illness but maladjustment, and not brain but mind.

Thus, D. B. Smith and his fellow editors have a difficult task. Their title is *Neurobehavioural Problems in Epilepsy*, and this immediately identifies their stance. No line is drawn, the issues of definition are not addressed, and the biological view of behaviour is assumed to encompass all. Here all behaviour is neurobehaviour. The book is thus strongest where the 'neurobehaviours' are most clearly organic, for instance in the symptomatologies of the seizures themselves (perceptions, affects, amnesias) or where epileptic neurobehaviours are based on physiological or pathological changes, where neurological aspects are discussed or where behaviour is scarcely discussed at all (for instance, in an excellent chapter on the histopathology of the temporal lobe). These sections of the book are uniformly excellent and informative. The book is weakest exactly where neurology is weakest, and where evidence for an organic process is poor. The assumptions that unitary hypotheses can explain disorders of affect or cognition in epilepsy seem futile; yet considerable research effort has been expended in exactly this arena. It surely surprises no one to recognize that mood is not resident in the frontal lobes, or that aggression is only due to dopamine excess, yet these beliefs are made implicit in some chapters in this book. The reverse problem that purely psychological 'treatment' can influence the organic basis of seizures is also considered in several chapters, again a largely invalid exercise. The problems of simplified psychological description of complex behaviour is perhaps most clearly illustrated in such subjects as the Geschwind Syndrome. It is worth quoting Benson at length, who tackles the problem of psychiatric classification head on, as this seems to me a distillation of the problem: 'The Geschwind syndrome remains questioned and uncertain as a specific entity . . . it is not common . . . many of the traits can be seen in non-epilepsy behavioural disorders. Nevertheless, the combination of several otherwise uncommon findings suggests a true syndrome.' To resolve the problem (*sic*), Benson discusses correlations and defines a further syndrome (the temporal hyperconnection syndrome, comprising hyposexuality, stickiness, cognitive intensification, emotional intensity and a seizure focus in the anterior temporal lobe). Benson's chapter is rigorous as one would expect from an excellent clinical scientist, but does such an approach, relying on the medical model, really advance knowledge further?

The volume was the result of a conference, with over 50 contributors, eminent in the field, and 450 pages of dense neurobehavioural argument. It is well supported by over 3000 references to the literature, and will be standard work in this murky field. Like all conference proceedings, it does not push forward the frontiers of research but serves rather to summarize current thought; and it succeeds well in this purpose. A good addition to the generally excellent *Advances in Neurology* series.

Perhaps the most interesting subject of all in the field of the neurobehaviour of epilepsy is that of the psychoses. It has been long observed that psychotic behaviour may occur in several epileptic states. An acute psychosis may occur during or after seizures, a chronic pyschosis may be present interictally, psychosis may occur after surgery, or due to drug treatment. Biochemical explanations of psychosis have been notably futile, anatomical explanations have been obsessed with essentially trivial notions of temporal lobe laterality, and physiological explanations tend to rely on the unreliable interictal scalp EEG. The subject is thus in some confusion. Michael Trimble's monograph *The Psychoses of Epilepsy* is a welcome addition, not least as it is the work of a single experienced author with a long-standing academic interest in this subject (not a manuscript prepared for a conference deadline!); it is well written, well researched and stylistically coherent, in short a book, and not another miscellany. The subject is approached historically, and Michael Trimble is very good on the development and intellectual evolution of the concepts of psychosis. This seems particularly valid when considering classification, and there are well-deserved assaults on the DSM tyranny which dominates modern psychiatry. The different psychoses of epilepsy are considered in turn, and particularly good is the chapter on post-operative psychosis, a considerable issue in modern epilepsy practice. The book provides an excellent review of the literature, but one slight regret is that more of Dr Trimble's own clinical material from the National Hospital was not introduced. In an appendix, it is noted that Van Gogh developed the Geschwind syndrome (with hypergraphia manifested by a prodigious output of painting, and no doubt also the features of temporal hyperconnectivity). He was considered by Jaspers to be schizophrenic, and by others to be syphilitic, alcoholic or poisoned by absinth. Dr Trimble, following Gastaut, argues coherently for an epileptic basis for his psychosis; Van Gogh, however, simply thought he was *sain d'esprit* (sane, not a DSM-111 category). Plus ça change . . .

SIMON SHORVON

Motor Control, Concepts and Issues. Edited by D. R. Humphrey and H.-J. Freund. *Dahlem Workshop, Life Sciences Research Report*, Volume 50. 1991. Pp. 500. Chichester: John Wiley and Sons Price £70.00.

It is a paradox that, given time, monkeys and typewriters locked away in a room together can come up with Shakespeare, but a group of scientists closeted away in a bunker in Berlin for a week come up with no new ideas at all. At least that is the impression gained from reading the considerations of the four committees which reviewed the 21 papers in this workshop. Pearls of wisdom such as, 'it is important to identify and spell out proper questions before correct answers can be found' (p. 118), or that 'progress will come from continued focus on functional questions' (p. 259), or my favourite, 'that the time is now ripe for a thorough reinvestigation of . . . various clinical motor syndromes' because 'new tools exist for . . . analysis of . . . movement' (p. 377), simply reflect the well-known fact that scientific progress is a very individual effort. Science by committee (and the research councils are no exception) is pedestrian.

I do not question the effort which the eminent people invited to the workshop put into their discussions. It is simply that the unwritten rules of these meetings stunt any sparks of innovation. When the members of each committee meet they all recognize the importance and urgency of their chosen subject, note a surprising degree of agreement about the need for both change and continuity, and emphasize the importance of still more communication and exchange of views at future meetings. No one must get angry or be destructive or negative, and no one must question the good faith of the sponsors and organizers.

Ironically the only report which showed any signs of life was the one which was most heavily biased towards the opinions of the man who wrote it, Dr S. Wise. Not only did he insist on inserting his own very useful diagram of equivalent nomenclatures for the motor cortical areas despite the specific instructions of the committee to set those issues aside and concentrate on functional questions, but he also broke the unwritten rules of conference proceedings by failing to come up with any suggestions for future research in his concluding paragraph. He, at least, realized that rules for research are boundaries: only new hypotheses carry us forward.

Although the committee reports gloss over the problems, there were indeed some divisions of opinion among the participants. It was only good manners, I am sure, that prevented Dr Macpherson's views on postural muscle synergies being torn apart by the Lundberg school, a powerful and extraordinarily clever group of scientists who work on the interneuronal circuitry of the spinal cord, who thought that they alone were entitled to use the term synergy, since it had been handed down to them in a direct line of descent from Sherrington. The Lundberg school were up in arms again listening to Kalaska's account of single cell recording in motor cortical areas, and the model of population vector coding as a tool for interpreting cortical discharge. I am a great admirer of this work, begun in the United States by Georgopoulos *et al.*, but I admit to being seriously swung by the electroanatomists. How could this man, they seemed to be saying, tell us how the brain works, when he is recording from unidentified cells, with inputs from unknown sources, and with axons that might project to any one of half a dozen different targets. They pointed out that the cells in motor cortex do not form an homogeneous population: some project monosynaptically to the spinal cord, some to the promoter areas, some to parietal areas, etc. So what is the rationale for believing that their average behaviour codes for anything meaningful at all?

New ideas, of course, will always be attacked, and where change is fastest old certainties disappear Indeed it is uncanny how messy the development of new ideas can be. Strick has discovered two new motor areas in the cingulate gyrus and sulcus which are separate from the nearby supplementary motor area. How does an anatomist know that these are motor areas? Simple, by proposing a new definition of motor areas which includes them. The new areas have projections to both the primary precentral motor strip, and to the spinal cord, so that this becomes the new definition. A circular argument, but necessary as a straw on which to grasp while ideas continue to circle.

Forget the committees. Read the individual contributions to this book to find out where new ideas really come from.

JOHN ROTHWELL

Perspectives on 'Hysteria'. By Philip Slavney. 1990. Pp. 220. Baltimore: Johns Hopkins University Press. Price £17.50

From Paralysis to Fatigue: A History of Psychosomatic Illness in the Modern Era. By Edward Shorter 1992. Pp. 419. New York: MacMillan. Price $24.95

Bertha Perlmann was a young hairdresser of 23, living in East Prussia, who had a 6 yr history of severe headaches, vomiting and pelvic pain. She had been advised by her local doctors in Koenigsberg, and the Professor of Surgery in Freiburg that her symptoms would be cured by an ovariotomy She had declined, but a trip to the spa brought no relief, and she became so weak any further travel was impossible. She was thus admitted to a Berlin Hospital, where her cervix was removed, but without effect. Three more physicians recommended ovariotomy, and she was finally referred to the brilliant young surgeon, James Israel, head of surgery at Berlin's Jewish Hospital. On December 31, 1879, he operated on her.

Dr Israel presented the results at a medical meeting in the following year. Bertha's vomiting had ceased, her pain had gone, and she felt well for the first time in 6 yrs. Israel continued: 'Now, gentlemen, this would have been a lovely cure of a difficult case of hysteria with a bilateral ovariotomy, if in fact I had done such an operation.' But he had not. All Dr Israel had done was simple skin incision under chloroform narcosis.

This example, chosen from many similar anecdotes to be found in Shorter's fascinating, scholarly and provocative book, illustrates the enduring capacity of hysteria to cause drama. Like all dramas, the story of hysteria comes in several acts, with this anecdote illustrating one particular scene change, the decline of the reflex theory of hysteria, which held that it was the result of reflex irritation of organs such as uterus or ovary. In its place came the central nervous paradigm, hysteria as a brain disorder. The history of hysteria is replete with similar examples of shifts and changes in the meaning of the term, from the ancient Greek wandering uterus to the modern, much heralded, but so far unseen, demise of the concept.

Doctors' views of what makes an illness 'psychosomatic' have always been, and show no signs of ceasing to be, based largely on culture, stereotype and prejudice. As such they prove irresistible to the historian. There are two approaches to the subject, the history of medical writings on hysteria, and the history of the patients, and their dealings with doctors. The former treats hysteria as part of the history of ideas, using medical writings on the subject as the object of study. This approach is exemplified by the brilliant series of essays by Mark Micale [see *History of Psychiatry* (1990), **1**, 33 – 124]. The alternative is to study the clinical realities of the patient and doctor, the patient's symptoms and the doctor's attempts to explain and alleviate them. This approach is followed by Slavney, a clinician, and Shorter, a historian.

Shorter's title, which will act as a red rag to a bull in certain circles, implies that hysteria is changing. This is not a new observation, and many have attempted to answer the question as to what has become of the 'grande hysterie' of the Salpetriere? Of course, as Critchley has shown, and anyone working at the National Hospital for Neurology and Neurosurgery, Queen Square, will confirm, he or she has not disappeared entirely, but nevertheless, such cases no longer occupy the centre stage of neurological demonstrations. Shorter traces the switch from the paralysed, convulsing hysteric of Vienna and Paris to the exhausted, depressed somatizer of St Louis. Certainly the change from paralysis to fatigue was dramatic, exemplified by the eclipse of hysteria by neurasthenia. Shorter makes many telling points about the reasons behind this shift in paradigms, such as new advances in neurophysiology, the discovery of the electrical nature of the nervous impulse and the impact of the Second Law of Thermodynamics. Diseases could now be seen in terms of diminished reserves of energy, to be restored by electricity, or conserved by rest. One other explanation is simply the natural shifts in fashion that accompany these conditions, usually triggered by a change in social status of those afflicted. Thus *The Spectator* of 1894 (**72**, 11–12) commented that.

> 'There was a time when hysterics were considered quite a proper and pleasing phenomenon among women. No lady who respected herself would ever have met with certain mishaps without immediately indulging in a fit of hysterics, or resorting to the gentle device of fainting. Now hysterics and fainting fits have become unpopular in the drawing-rooms of society, and are rarely met with except in the kitchen. Fainting and hysteria were once considered rather interesting, and weak nerves seem now to be regarded in the same light.'

It is no wonder that this was written at the time of another shift, when weakness of nerves, neurasthenia, replaced 'hysteria'. The subsequent decade was dominated by the figure of the 'New England Invalid', Proustian figures stretched on their chaise-longues. Unfortunately, this shift, whilst avoiding the new stigma of hysteria, was not always accompanied by similar advances in treatment. Shorter does not quote Dutil's 1904 description of the consequence of the fashionable treatment for neurasthenia, the rest cure, which resulted in 'the patient, condemned to complete inactivity, lying on her chaise-longue for days and nights . . . constantly alert to the most minute sensations, surrounded by those excessively concerned for her health'. It was the failure, and expense, of these approaches, and the apparent success of the new psychotherapies that led to the eclipse of neurasthenia, and the emergence of yet another paradigm. For a while psychoanalysis became the great hope, although ironically it too attracted the same criticisms of the rest cure; it was doubtful if it worked, but it certainly was expensive.

The next change was also of venue, as the story moves from Boston, Paris and Berlin to St Louis, the new home of somatization disorder as we must learn to call it. Shorter, the non-clinician, is more inclined to accept this latest formulation at face value, but as Slavney points out, somatization disorder is almost as elusive a beast as plain old hysteria. Their desire to reify it, by producing operational criteria, prognostic data and even genetic studies, does not blind Slavney to the deficiencies of the concept. For example, he points out that if you define a disorder as chronic, the fact that on follow-up the condition appears stable, proves very little.

What do we learn from this story? One theme constant to both books is the ever-present stigma of emotional disorder. Neurosis is fine so long as it is seen as a physical disease of nerves. Hysteria is fine so long as it is a disorder of the brain. However, the success of hypnosis and suggestion, which predates Freud, starts to cast doubt on this. Imperceptibly Charcot, and thus most neurologists, move hysteria towards the rest of mental illness, until, in our own time, no one believes it to be organic any more, and in both medical and popular usage hysteria becomes shorthand for all mental disease, the product of the imagination

It was thus inevitable that as the organic view of hysteria weakened, the term became one of reproach. The Victorian response was to develop a series of diseases indistinguishable from hysteria except that the sufferer was male, or, if female, of impeccable standing, and the cause organic. In Hack Tuke's dictionary of 1892 it was explained that one of these illnesses, neurasthenia, could be diagnosed when hysteria might seem insulting, 'especially by those who confuse hysteria with malingering'. The difference was that the neurasthenic gave 'all they possessed to be well, and heartily long for good health if only they knew how to obtain it. A conditions such as this, in such women, is as far removed as possible from hysteria'.

Shorter outlines a series of illnesses which fulfil the same role as neurasthenia, including chronic brucellosis, hypoglycaemia, total allergy syndrome and, more controversially, the current epidemic of chronic fatigue syndrome, known in this country as 'ME', the common acronym for myalgic encephalomyelitis. He is correct, not in the sense of these conditions being new names for hysteria, which they are not, but as the natural home for those who, like the Victorian neurologists, are uncomfortable with the psychological in any disease. In a recent newsletter, the current President of the ME Association wrote that it was an outrage to call ME hysteria. Indeed it is, but the words would not have been out of place in the *Spectator* of 1894. Myalgic encephalomyelitis could not be hysteria because sufferers are 'hard working, disinclined to rest, and frequently conceal the illness in order not to let down colleagues and relatives, hardly the picture of hysterical exhibitionism or mental derangement'. The same doctor told a journalist that ME could not be depression either, because ME patients have 'too much will power whereas depressives have virtually none'. The doctor was correct to state that ME is not hysteria, nor is it solely depression, but it is the evidence chosen to make this point that is so revealing. Just as in the Victorian era, mental illness remains a moral judgement, associated with a failure of will-power.

Shorter shows how neurasthenia gradually suffered the fate of hysteria, as its organic basis, as well as restriction to the better classes of society, was questioned. The turn of the century neurologist was faced with a fundamental problem: what to do with the neurotic patient. At that time the care of the emotionally distressed but non-insane patient was almost entirely the province of the neurologist, since neurological private and outpatient practice was founded on the care of the neurotic patient—as, the cynic might add, it has remained. For a while neurologists resisted the new Freudian ideas (too foreign, and too sexual). However, given that it was becoming increasingly hard to view these illnesses as organic, the only alternative aetiologies, such as low moral fibre, or feminine traits, were little better. The solution, quickly adopted in England, but more slowly elsewhere, was to stop seeing these patients (at least, when recognized as such). The beneficiaries were the psychiatrists. Whether or not this change was beneficial to the patient is not as clear-cut as it might appear. Shorter assumes that psychiatrists are the natural home for the depressed and neurasthenic, yet just as the old neurologists were 'mindless', the new psychiatrists were equally inappropriately 'brainless' Occasionally, Shorter loses sight of the fact that we remain in ignorance of the true nature of many of the conditions under consideration, and he is uncritical in his assumption that all these illnesses are indeed psychosomatic, as seems unlikely. For example, the Victorians knew from careful clinical observation that neurasthenia frequently followed an infective illness. This insight was forgotten (perhaps because the association could not, and still cannot, be satisfactorily explained) but the oversight has had unfortunate consequences.

There was another problem with hysteria, to which both Slavney and Shorter devote attention. It was predominantly a disorder of women, just as somatization disorder is today. In Janet Oppenheim's words, it was the 'archetypal female disorder'. Many feminist historians have made much of this, as well they might, and Slavney agrees with those who claim that in the hysterical personality disorder psychiatrists are diagnosing a caricature of femininity, rather than a disease. Such analyses are, of course, sound, but are not the complete picture. Hysteria was a disease of women, but doctors were not as reluctant to diagnose it in males as some historians have claimed, and those who read the current journals will have noted two recent articles on somatization disorder in men.

Acknowledgment of the existence of male hysteria was begun by Charcot, a muted figure in Slavney's pages, but who comes alive in Shorter's account. For a while English neurologists avoided the lessons of Charcot's teaching because he was, after all, French. Thomas Dowse wrote in 1889 that 'the Gallic nature seems to be of less enduring stability than that of the Saxon, and is more liable to exhibit hysterical manifestations'. Not only women were liable to hysteria, but excitable foreigners as well. However, the flood of 'shell shocked' soldiers returning from World War I meant that it was impossible to deny that men, even English ones, could also develop psychological disturbance under stress. Even then, officers were more likely to be diagnosed as neurasthenic, and sent to sanatoria, whilst the rank and file, diagnosed as hysterical, were treated less sympathetically. The study of hysteria thus illuminates not only medical stereotypes of women, the usual end-point of the feminist reading, but all those thought to be morally weak, which includes all those with emotional disorders, or even anything that does not conform to ideas of legitimate disease.

Shorter argues that the diagnosis of hysteria, somatization disorder, neurasthenia and its modern equivalents is as much a cultural and moral exercise as a medical one. However, as both he and Slavney are in no doubt, the phenomena covered by these terms are real and tangible, however flawed and occasionally absurd may be our attempts to pigeon-hole them. Patients present to doctors with inexplicable symptoms, demanding help. Doctors cannot escape their duties by merely saying 'you are a cultural phenomenon'. Action is required.

It is recognition of the pressures on the doctor, as much as the patient, that characterize the latest change in our conceptions of such illnesses. Shorter and Slavney both recognize the two strands to the story: the patient's narrative, and that of the doctor, although here Shorter's historical perspective is more illuminating. Slavney, however, introduces the sociological concept of the sick role: how people behave, how they are expected to behave, the rewards from family, friends, doctors and employers if they do, and the adverse consequences if they do not. As he shows, this represented another, albeit useful, shift in our understanding of hysteria, but still questions remain unanswered. We know that clinically one group at high risk of pseudoseizures are those with pre-existing epilepsy. But why should they use hysteria to lay claim to the sick role, when they possess it already by virtue of their epilepsy? Nevertheless, the advance represented by the new sociological constructs such as the sick role is to bring the observer into the frame in a way that previous formulations failed to do.

Like all dramas, hysteria must have an audience. Slavney grasps that hysteria is not the same as other diseases that one simply has, and instead describes it as something one is. To prove this point he makes use of studies of hysterical traits to illustrate relatively stable lifelong traits. However, even if the traits of self dramatization and emotional lability are reliably recorded, and relatively stable, is that hysteria? I suspect not. Hysteria is not something that can be limited to a single person, either briefly (something one has) or enduringly (something one is). Slavney sees self-dramatization as an enduring trait of the hysteric, but, unlike Shorter, misses the need for the audience. Without an audience there is no drama. That audience is provided partly by family and friends, but most of all by doctors.

Persistent hysteria cannot happen without validation by a medical authority. David Taylor has outlined the often extraordinary lengths that people, all too often parents, will go to find the doctor who will endorse their belief. Only one is necessary, and once that prophet has been found, all the scepticism of the rest merely reinforces the status of the 'good' doctor who understands about the allergy that prevents the child from walking, the chemical sensitivity that confines him to bed, and the virus that destroys the immune system. The infliction of illness onto another has now been recognized by yet another label: Munchausen's by Proxy, but the essential theatre, accompanied by the usual media spectators, remains unchanged.

For as long as there have been recognized diseases, there has been hysteria. Of course, in every generation diseases thought to be hysterical are removed from the register, admitted to the pantheon of 'real' diseases, and their previous adherents mocked. Elliot Slaters famous warning is thus a salutary one, but should not be taken too far (and we must remember that an opposite process occurs as well, as previous legitimate passports to medical care lose their credibility). When people in distress are denied the sick role, they will continue to seek it, and to seek doctors who will give them what they desire. Each new International Classification of Diseases will seek a new descriptive label, but the process will remain.

Thomas Szasz, who has also declared hysteria dead, wrote that 'the hysteric is a liar who does not admit or recognize his lie'. Deceit and deception figure prominently in the medical conceptions of hysteria. Both authors outline how hysteria changes from being a cause of other illnesses, via mimic of other illnesses, to the hysteric as a mimic. The main preoccupation of the modern neurologist is to catch this deceit, whether by the disease or the patients. The clever neurologist is alive to the possibility of an unusual presentation of rare neurological disorders, and if the attempted deception by the disease is sufficiently unusual, and the doctor sufficiently ambitious, a paper will result to record the skill of the doctor in detecting it. On the other hand, the neurologist also has several tricks of the trade to catch the deceptions by the hysteric: letting the hand fall above the head, watching for agonist and antagonist muscle contractions, and delineating the impossible sensory level. As Dr Israel showed when presenting Bertha Perlmann, hysteria encourages flamboyance and the conjurer's art in doctor and patient.

Psychiatrists are also not above flamboyance and collusion Peter Gay describes how the most famous shift in the story of hysteria was initiated by the epoch-making collaboration between a gifted patient, Anna O., and her attentive physician, Breur. The modern psychiatric equivalent of Charcot's Tuesday lessons with their carefully rehearsed dramas may be the current vogue for multiple personality disorder. American clinicians specializing in this disorder have been maliciously, but perhaps not inaccurately, described by a recent correspondent as 'never missing an opportunity to show photographs, movies or videos of their uniquely talented offspring or to tell you of their latest cute trick' [*British Journal of Psychiatry* (1989), **155**, 565]. Perhaps there is something in national characteristics after all, for just as we were relatively immune to Charcot's grande hysterics, we also appear immune to multiple personality disorder as well.

In the last analysis hysteria may not be, in Slavney's words, something one is, but something one becomes. Many illnesses labelled hysterical do not start that way, and may commence for a variety of reasons, each of them organic enough to satisfy even the most sceptical neurologist. However, as neither author acknowledges in sufficient depth, what starts an illness may not be what sustains it, and other dramas and meanings can attach themselves with the passage of time. Hysteria begins once illness becomes an escape from unacceptable situations. Fight or flight is the classic response to danger, but hysteria may provide another escape from situations in which a person's psychological integrity or views of himself or herself are challenged. Sadly, once danger is passed or defeated, a person can stop running or fighting, but symbolic illnesses, once begun, are harder to abandon.

Fanciful? the *Evening Standard* ('Malingerer or Miracle Cure': November 21, 1991) recently carried a detailed story on a lady, who, after 8 yrs confined to a wheelchair, fully validated by her doctors and the DHSS, was cured overnight by faith healing. She has written a book about her experiences. Many of her neighbours, originally so supportive and kind, now shun her in the street, judging that she was a hysterical malingerer all the time. Her self-help organization is embarrassed, and even the DHSS is reluctant to accede to her request to stop all her benefits. Her efforts to change the script of her illness were thus resisted by family, friends, doctors and administrators, demonstrating just how hard it is to relinquish the sick role, and to end a story happily As both doctors and patients have discovered, ending hysteria is always more difficult than it seems, and one thus doubts Slavney's own claim that his will be the last book that includes hysteria in its title.

SIMON WESSELY

150 Years of British Psychiatry 1841–1991. Edited by G. E. Berrios and H Freeman. 1991 Pp. 464. London: Gaskell, Royal College of Psychiatrists. Price £15

Published to celebrate the 150th anniversary of the Association of Medical Officers of Asylums and Hospitals for the Insane and its successor the Royal College of Psychiatrists, this book reviews the people, ideas and institutions which have shaped modern British Psychiatry. Its eclecticism is illustrated by the inclusion of two chapters on the history of Irish psychiatry, as well as psychiatry in colonial India and in Scotland. Another welcome feature is the departure from the many histories of psychiatry which marshall historical arguments into the service of the polemic. Foucault's *Madness and Civilization* and Skull's *Madhouses, Mad-doctors and Madmen* are some of the more famous examples of this school

Instead Berrios and Freeman do not appear to have selected their contributors with any consistent conjecture in mind, though the contents are perhaps dominated by psychiatrists with an interest in history over historians studying psychiatry. Other omissions include the absence of attempts to chronicle patients' views over this time, or to represent the voice of women. Peterson's *A Mad People's History of Madness* and Ripas' *Women and Madness* remain lonely contributors to this area. Yet it is easy to find excluded areas in histories, and this book succeeds in being as comprehensive as a similar venture—*The Anatomy of Madness* by Bynum, Porter and Shepherd—but is also more affordable. Whilst the roots of modern British psychiatry, from psychosurgery to psychotherapy, are dug up with admirable gusto, it is true to say that this Victorian era of psychiatry may have now been over-farmed. Titles covering this period seem to appear with monotonous frequency. They include, Busfield's *Managing Madness,* Leigh's *The Historical Development of British Psychiatry,* Arieno's *Victorian Lunatics* and Donnelly's *Managing the Mind.*

There has been much less published on the period before the 1800s, Porter's *Mind-Forg'd Manacles* and Hunter and Macalpine's *Three Hundred Years of Psychiatry* serve as reminders of how fertile these other periods should be if properly investigated. Of course, the editors focused on this age as the book is intended to probe the influence on the history of psychiatry of the Association and the Royal College.

G. M. Young suggested that it is the historian's job to study his subject until he can hear the people speak and this book contains a veritable babble from the history of the National Health Service to the history of the community, from the history of the text-book to the history of anti-psychiatry.

It is interesting to compare this celebratory work with a similar attempt by the North Americam profession which produced *American Psychiatry Past, Present, and Future* in 1975 to commemorate the establishment of the first state-supported mental hospital in America, edited by Kreigman, Gardner and Abse. The British book is more scholarly and evocative, but does omit a serious discussion of the future of British psychiatry, given the past. There is thus little attempt to draw the various themes together, unlike the United States volume which concluded with a discussion of the current place of psychiatry in society and its future direction. This tentativeness may betray some of the apologistic tendency in historical studies, which unconsciously feel inferior to high technology medical research at the interface with serious diseases. This is unnecessary, as the history of a science is the science itself.

RAJENDRA PERSAUD

Towards a New Pharmacotherapy of Pain. Edited by A. I. Basbaum and J. M. Besson *Dahlem Workshop, Life Sciences Research Report.* Volume 49. 1991. Pp. 457 Chichester: John Wiley and Sons. Price £65.

The Dahlem Conference in Berlin is a unique forum for analysing, in a multidisciplinary way, complex topics. For 5 days 50 selected participants are cloistered together, divided into four groups. Each group studies background papers prepared by a few selected individuals which serve as a basis for further discussion and the preparation of a report. The goal of these reports is to define what is not known in the field rather than rehearsing what is known and to present ideas for further research and their priorities. This volume represents the product of such a conference held in November 1989 whose stated goal was 'to better understand nociceptive mechanisms and to explore new strategies for the design of analgesic drugs that will support or enhance the use of morphine'

Although these conferences are famous, or notorious, for the thoroughness of the planning and organization, what could not have been anticipated was that at the exact time of the conference, the Berlin Wall would begin to crack and then collapse under the weight of individuals, sick with the failure of ideological despotism in the East. A feature of science in the Eastern Block was science by committee. It is therefore ironic perhaps that the absolute highlights of this book are not the individual background chapters, worthy as they are in most cases, but the four group reports, written under the guidance of a rapporteur and hammered together into final form by a complex dialectical process that is explained in the foreword. These group reports are essential reading for all interested in pain and its mechanisms. They constitute a summary of key controversies, technical and clinical problems and guesses about future developments. Unfortunately, they do not provide immediately any new pharmacotherapy of pain nor even a promise of any new therapy. What they do contain is a fascinating dialogue between clinicans, basic scientists and representatives of the pharmaceutical industry.

This is the second Dahlem conference devoted to the topic of pain. The first, held a decade earlier, now seems somewhat obsolete. Substance P was thought then to be *the* pain transmitter and therefore an appropriate antagonist would be the perfect analgesic; endogenous pain control systems operating via the endorphins and enkephalins were thought to offer the possibility of a controllable endogenous analgesia; and finally morphine was demonstrated to act at discrete receptors at spinal and supraspinal sites introducing a new site-directed narcotic treatment. The latter finding has contributed enormously to pain management, by enabling high dose intrathecal or epidural narcotics to be administered with less risk of side-effects than systemic narcotics. The first was simply wrong, substance P is not the pain transmitter; indeed its role still remains elusive, and as for endogenous analgesia, the stimulation of descending pathways in humans is now widely accepted to be an ineffective or impracticable form of pain therapy. What will our view be of the present Dahlem Conference Proceedings, in 10 yrs time? It too will no doubt be obsolete but it will have served as a marker of enormous and vitally important changes in pain research, the realization that clinical pain results from central as well as peripheral mechanisms and that the conventional strategy of drug companies for the development of new analgesic agents has to be changed completely.

The first of the four group reports is entitled 'Discussion on the Physiological and Pharmacological Basis of the Transmission and Control of Nociceptive Messages', with Donna Hammond as rapporteur, and briefly but succinctly surveys the issue of what criteria need to be met for animal models of nociception, what are the roles of different peripheral nociceptors in generating different types of pain, what are the criteria for defining the involvement of a substance in nociception both in the periphery and in the central nervous system?

The second group report with Howard Fields as rapporteur is concerned with strategies for improving pharmacological approaches to the maintenance of analgesia in chronic pain. This group dealt with the ongoing controversy of the role of opioids in neuropathic pain, the significance of tolerance in limiting opiates in chronic pain management, together with a review of unresolved issues in the mechanisms of opiod analgesia. The report ends by stating that patients with chronic pain can frequently be expected to achieve considerable and prolonged pain relief with opioids and that while tolerance need not be a problem the reasons for treatment failure reflect the possible involvement of multiple mechanisms. The hope is expressed that further understanding will result in the prevention of pain transduction at the source of any abnormal message, rather than by interrupting central transmission with narcotics

The third report, with R. Zukin as rapporteur, considered 'What is the Molecular Basis of Opioid Antinociception and How Does this Information Point to New Drug Design?' The group dealt with the classification of opiod receptor subtype, bemoaned the failure of molecular biology to clone any opioid receptors, analysed whether particular opioid receptor subtypes can be attributed with anti-nociceptive effects at particular sites, whether endogenous peptides are important for nociceptive transmission and therefore if drugs acting by inhibiting specific peptidases are suitable candidates for analgesics and finally whether modern medicinal chemists can construct suitable peptidomimetics for opioid receptors.

The final report with Marshall Devor as rapporteur attempts to define the mechanisms involved in generating neuropathic pain following peripheral nerve injury. This group concentrated on the relative peripheral and central changes that may contribute to these chronic pain syndromes including the involvement of the sympathetic system. How the pain is initiated and how it is maintained were discussed. Several hypotheses were proposed emphasizing the relative importance of long-term central changes (central sensitization). The relevance of these potential mechanisms to the pharmacotherapy of neuropathic pains was also discussed, including the possible advantage of vigorous early treatment. Once again the problem of the appropriate laboratory model for studying the sensory changes in man associated with nerve injury, rears its ugly head.

Together these four chapters, although only about 25% of the volume, offer a fascinating insight into current research in the field of pain. They are honest about current shortcomings in our knowledge and provocative about what mechanisms may operate and how they could be tested. The rest of the volume contains many excellent chapters. One that reminds us of the enormous difference between the interests of academics and the pharmaceutical industry is that of Ray Hill describing how the industry views the development of analgesic drugs What is clear is that a sufficiently large potential population is the key factor and therefore low back pain is targeted but not patients with reflex sympathetic dystrophy. Another chapter worthy of special mention is that entitled Nociceptive Models: Relevance to Clinical Pain States by Ward, Portenoy and Yaksh, which argues that the classical models of nociception may not always predict whether particular drugs will produce analgesia in man. A pathophysiological classification of clinical pain is presented together with categories and descriptions of experimental pain/nociceptive paradigms. When combined together a relationship between different experimental models and different pain classes with different physiological and neurochemical correlates can be drawn up. Although I do not agree with all of their analysis, this approach reflects a change in our thinking about pain mechanisms and leads inescapably to the conclusion that what most basic scientists and pharmaceutical companies have been studying for the past 50 yrs has little or nothing to do with the clinical problem of pain.

We live in exciting times. The literal breakthrough of the Berlin Wall at the time of this symposium coincides with breakthroughs in our understanding of pain mechanisms. Our current approach is getting more sophisticated as we unravel the complexities involved. The way ahead does offer the possibility of a new pharmacotherapy of pain. It will almost certainly be different from that envisaged at this meeting and opioids will be difficult to supplant but this volume has helped by clearly defining where we have got to, even if that is not as far as we would like to be. However, in the short time since the meeting tremendous progress has been made, non-peptide tachykinin antagonists have been developed, narcotics have been demonstrated to produce analgesia when administered peripherally and the involvement of the *N*-methyl-D-aspartate receptor in central sensitization documented. Pain research, after a relative lull at the beginning of the 1980s, is on the move again.

CLIFFORD J. WOOLF

Dystonia Myotonica and Related Disorders

Admirers of the classical monograph by Caughey and Myrianthopoulos, *Dystrophia Myotonica and Related Disorders* (1963), will be delighted to learn that it has been reprinted. The original book provided excellent accounts of the clinical aspects of the disease and its endocrine and skeletal manifestations. Now these are supplemented by additional chapters on the Greek Ypsilante family and genetic advances. Copies may be obtained from The University Book Shop, PO Box 6060, Dunedin, New Zealand. The price is NZ$39.60 plus postage.

ANNOUNCEMENTS

Courses

The following course is being organized by the Institute of Psychiatry (University of London), the Maudsley Hospital and Bethlem Royal Hospital:

'Treatment and Care in Old Age Psychiatry', July 8–10, 1992.

For further information contact Mrs Lee Wilding, Conference Office, Institute of Psychiatry, De Crespigny Park, London SE5 8AF (Tel: 071 703 5411, ext 3170; Fax· 071 703 5896).

The Medical College of Wisconsin and Continuing Medical Education, Inc. announce the seventh in a series of courses: 'The Sharper Image in MRI' MR Angiography and Imaging of the Head, Spine and Musculoskeletal System, July 27–31, 1992, at the Broadmoor Resort in Colorado Springs, Colorado, USA. Fees: $495.00 before June 5, 1992, $545.00 after June 5, 1992 Contact: Marti Carter, CME, Inc., 11011 W. North Avenue, Milwaukee, WI 53226, USA (Tel: 414 771-9520).

Conferences organized by The New York Academy of Sciences

'The Neurohypophysis: a Window on Brain Function' at Dartmouth College, Hanover, New Hampshire, USA, July 16–20, 1992

'The Melanotropic Peptides' at Palais des Congrès, Place de la Cathédrale, Rouen, France, September 6–9, 1992.

'Symposium on Temporal Information Processing in the Nervous System with Special Reference to Dyslexia and Dysphasia' at Vista Hotel, New York City, USA, September 13–16, 1992.

'The Role of Insulin-like Growth Factors in the Nervous System' at Crystal Gateway Marriott, Arlington, Virginia, USA, November 4–7, 1992

For further information contact the Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA (Tel: 212 838-0230; Fax: 212 888-2894)

The Ninth International Congress of Electromyography and Clinical Neurophysiology

This congress will be held in Jerusalem, Israel, June 8–12, 1992. For information contact Dr Arieh N. Gilai, Convener, EMG-CN-IX Congress, PO Box 29334, Tel Aviv 61292, Israel (Tel: 972 3 5102542; Fax. 972 3 660604)

The Thirty-second International Neuropsychiatric Gerald Grinschgl Symposium

This symposium will be held in Graz, Austria, June 14–21, 1992. For information contact Professor DDr.h.c Helmut Lechner, University Department of Neurology, Auenbruggerplatz 22, 8036 Graz, Austria (Tel: 43-316-385 2385; Fax: 43-316-35 520).

The Second International Congress of Movement Disorders

This congress, jointly sponsored by the International Medical Society of Motor Disturbances and the Movement Disorder Society, will be held in Munich, Germany, June 24–26, 1992. For information contact Secretariat, ISMD, PO Box, CH-4005 Basel, Switzerland (Tel. +61 691 51 11; Fax: +61 691 81 89)

The Third Meeting of the European Neurological Society

This meeting will be held in Lausanne (Palais de Beaulieu), Switzerland, June 27 to July 1, 1992. For information contact Sabine Geiger, Administrative Secretary ENS '92, c/o AKM Congress Service Clarastrasse 57, PO Box CH-4005 Basel, Switzerland (Tel: 41-61-691 51 11; Fax: 41-61-691 81 89).

The Eleventh Annual Scientific Meeting and Exhibition of the Society of Magnetic Resonance in Medicine

This meeting will be held in Berlin, Germany, August 8–14, 1992. For further information contact SMRM, 1918 University Avenue, Suite 3C, Berkeley, CA 94704, USA (Tel· 510 841-1899; Fax: 510 841-2340)

Ultrasound Angiography 92—2nd International Conference

This conference will be held in the Medical and Biological Sciences Building, University of Southampton, UK, September 2–4, 1992 For further information contact Conference Secretariat, Ultrasound Angiography 92, PO Box 15, Eastleigh, Hampshire SO5 5XG, UK.

The Second International Congress on Objective Assessment in Rehabilitation Medicine—Low Back Pain and Brain Injury

This congress will be held in Montréal, Québec, Canada, October 5–6, 1992. For further information contact Centre de formation en réadaptation du Québec, 6300 Avenue Darlington, Montréal, Québec, Canada H3S 2J4 (Tel 514 340-2089, Fax: 514 340-2149).

AAEM 1992 Annual Meeting

The Thirty-Ninth Annual Scientific Meeting and the Fifteenth Annual CME Courses of the American Association of Electrodiagnostic Medicine (AAEM) will be held in Charleston, South Carolina, USA, October 14–18, 1992 For information contact The AAEM Office, 21 Second Street SW, Suite 306, Rochester, MN 55902, USA (Tel. 507 288-0100; Fax· 507 288-1225).

The Eighth Conference of the South East European Society for Neurology and Psychiatry

This conference will take place in Jerusalem, Israel, February 28–March 3, 1993 For information contact Professor S. Feldman, The VIII Conference of the South East European Society for Neurology and Psychiatry, c/o PO Box 50006, Tel Aviv 61500, Israel (Tel: 972 3 5174571, Fax· 972 3 655674)

Second International Conference on Stroke

This conference will take place in Geneva, Switzerland, May 12–15, 1993. For information contact N M. Bornstein, MD, International Conference on Stroke, c/o Kuowi Travel Ltd, 7 Rue de Berne, CH-1211, Geneva 1, Switzerland (Tel 41 22 732 0888; Fax 41 22 731 5078).

The Ninth International Symposium of Brain Edema

This symposium will be held in Tokyo (Pacifico Yokohama), Japan, May 16–19, 1993. For information contact Umeo Ito, MD, Department of Neurosurgery, Musashino Red-Cross Hospital, 1-26-1, Kyonan-cho, Musashino-shi, Tokyo 18, Japan (Tel: 81-422-32 3111; Fax: 81-422-32 3525)

The Sixth Congress of the European College of Neuropsychopharmacology

This congress will take place in Budapest, Hungary, October 10–14, 1993 For information contact Peter Gaszner, Hüvösvolgyi út 116, Budapest, H-1021, Hungary *or* Congress Bureau MOTESZ, Ms Krisztina Szigedi (Tel: 361 251-7999, 163-0037, 163-3899, Fax: 361 183-7918).

Foundation Fyssen 1992 International Prize

The deadline for nominations for this prize is September 1, 1992 Subject considered elementary mechanisms of cognitive development For information contact Colette Leconte, Executive Secretary, Foundation Fyssen, 194 rue de Rivoli, 75001 Paris, France (Tel. 33 (1) 42 97 53 16; Fax 33 (1) 42 60 17 95)

Errata

Brain, Volume 114, Part III, June 1991 (pp. 1115–1127)

THE ROLE OF THE RIGHT HEMISPHERE IN EMOTIONAL COMMUNICATION

by LEE XENAKIS BLONDER, DAWN BOWERS *and* KENNETH M. HEILMAN

There were some minor errors in some of the means and SDs reported in Table 4 (p. 1122) of the above article. The ANOVA reported in the paper (Effect of Lesion Locus, pp. 1121 and 1122) is correct (it was computed from the correct values), so that these errors in no way alter our results. The revised table is given below.

TABLE 4 MEANS AND SDs ON TASK 2 VERBAL DESCRIPTORS OF NONVERBAL EXPRESSIONS

	RHD (*n = 10*)	*LHD* (*n = 10*)	*NC* (*n = 10*)	*All subjects*
Facial descriptors	71.3 (15.4)	79.5 (11.4)	85.8 (11.0)	78.9
Prosodic descriptors	71.5 (16.7)	85.8 (10.2)	93.7 (7.7)	83.7
Gestural descriptors	78.3 (9.7)	84.5 (7.2)	87.7 (7.5)	83.5
Overall mean	73.7	83.3	89.1	82.1

Effect of lesion locus. F(2.27) = 7.19, $P < 0.003$.

Brain, Volume 114, Part VI, December 1991 (pp. 2555–2573)

THE ROLE OF SENSORIMOTOR EXPERIENCE IN OBJECT RECOGNITION

A CASE OF MULTIMODAL AGNOSIA

by ANGELA SIRIGU, JEAN-RENÉ DUHAMEL *and* MICHEL PONCET

Two corrections were inadvertently omitted at the proof stage. The publishers regret any inconvenience caused.

The affiliation given for Michel Poncet was incorrect. His current affiliation is Service de Neurologie et Neuropsychologie, CHU de la Timone, Marseille et GDR-978, CNRS, France.

The legend to Fig. 4 (p. 2565) should read:

Sample items for the anomalous objects test. Clockwise from top left: 'economical faucet', 'bent hammer', and 'coffee pot for masochists'. (Copyright J. Carelman, reproduced with permission.)

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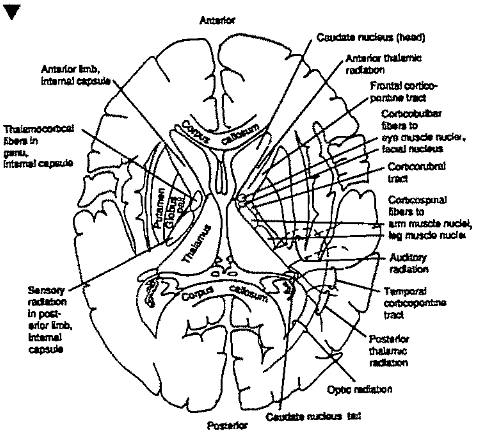
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Anterior thalamic radiation
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Corticobulbar fibers to eye muscle nuclei, facial nucleus
Thalamocortical fibers in genu, internal capsule
Corticorubral tract
Corpus callosum
Putamen
Globus pall.
Thalamus
Corticospinal fibers to arm muscle nuclei, leg muscle nuclei
Auditory radiation
Sensory radiation in posterior limb, internal capsule
Temporal corticopontine tract
Corpus callosum
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R. Llinás, C. Sotelo (Eds)

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P. Ascher, D. W. Choi, Y. Christen (Eds.)

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1991. XII, 155 pp 44 figs. (Research and Perspectives in Neurosciences) Hardcover DM 78,- ISBN 3-540-54134-9

This book, the first in the new series **Research and Perspectives in Neurosciences,** contains the proceedings of the meeting which was held in Paris on September 24, 1990. The series will deal with the most current research. Glutamate - the subject of the first volume - is an excellent example since it has a major role in many physiological or pathological situations.

S. L. Erdö (Ed.)

GABA Outside the CNS

Proceedings of the International Symposium on Peripheral GABAergic Mechanisms, Rome, July 19-21, 1991

1992. XIV, 298 pp. 62 figs 32 tabs Hardcover DM 164,-
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A. Arduini

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1991. XII, 251 pp Hardcover DM 148,-
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Dexter DT, Carayon, A, Vidailhet M, Ruberg M, Agid F, Agid Y *et al* (1990) Decreased ferritin levels in brain in Parkinson's disease. *Journal of Neurochemistry,* **55,** 16–20.

Dubowitz V (1978) *Muscle Disorders in Childhood. Major Problems in Clinical Pediatrics,* Volume 16 London. W. B. Saunders, p. 183

McGeer PL, Itagaki S, Akiyama H, McGeer EG (1989) Comparison of neuronal loss in Parkinson's disease and aging. In *Parkinsonism and Aging. Aging,* Volume 36 Edited by D B Calne, G. Comi, D. Crippa, R. Horowski and M. Trabucchi. New York· Raven Press, pp 25–34

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VILIUISK ENCEPHALOMYELITIS IN THE IAKUT PEOPLE OF SIBERIA

by L. G. GOLDFARB *and* D. C. GAJDUSEK

(*From the National Institutes of Health, Bethesda, USA*)

SUMMARY

Viliuisk encephalomyelitis (VE), a progressive neurological disorder with a fatal outcome usually in several months to 6 yrs after disease onset, is seen only among the Iakut people of Siberia The acute meningo-encephalitic phase of the disease is followed by progressive dementia, rigidity and spastic tetraparesis. The disease is characterized by multiple micronecrotic foci with marked inflammatory reactions and gliosis in the grey matter. The acute febrile onset, with cerebrospinal fluid (CSF) pleocytosis and increased protein in the CSF, the epidemiology and the inflammatory neuropathology suggest the disease is infectious. Studies on household spread indicate an incubation time of up to several years. Viliuisk encephalomyelitis was restricted to an ethically distinct group of Iakut people of the Middle Viliui region, but in recent decades, with migration from this region, it has been spreading into previously unaffected Iakut populations. The occurrence of multiple VE cases in households and introduction of the disease by migrants into new populations indicate horizontal transmission in a setting of long intimate contact.

INTRODUCTION

Viliuisk encephalomyelitis (VE) was first recorded a century ago (Maak, 1887) in a small rural population of about 4500 Iakuts on the left bank of the middle reaches of the Viliui River (Fig. 1). In the 1920s, VE was again reported from this region (Kolpakova, 1930). The disease has been recognized by the Viliui Iakut for over a century and is called by them 'bokhoror', a word for stiffness in the Iakut language. With population movement in World War II, the disease spread and between 1952 and 1958 many cases were found in neighbouring previously VE-free areas up and down the Viliui River, populated by Iakuts of a different genetic origin (Tokarev, 1940; Petrov, 1964). Since 1960, with further movement of population, VE has spread into Iakut populations in the densely populated Lena and Aldan Valleys, where more than 200 000 Iakuts reside (Goldfarb *et al.*, 1972*a*; Petrov, 1973; Gajdusek and Goldfarb, 1991).

Scientific study of VE began in the 1950s, when Shapoval and Sarmanova (1955), Petrov (1958, 1964) and Shapoval (1959) described the clinical features of the disease, and Sarmanova (1960) and Sarmanova and Chumachenko (1960*a*,*b*) attempted to discover its cause. In the 1970s, Chumakov *et al.* (1971) reviewed the VE literature and organized an interdisciplinary team of investigators to study the disease. A VE neurological clinic in Iakutsk and a home for chronically disabled VE patients in Viliuisk were established and used as bases for VE studies. The intensive studies reported here were carried out during the 10-yr period from 1970 to 1980.

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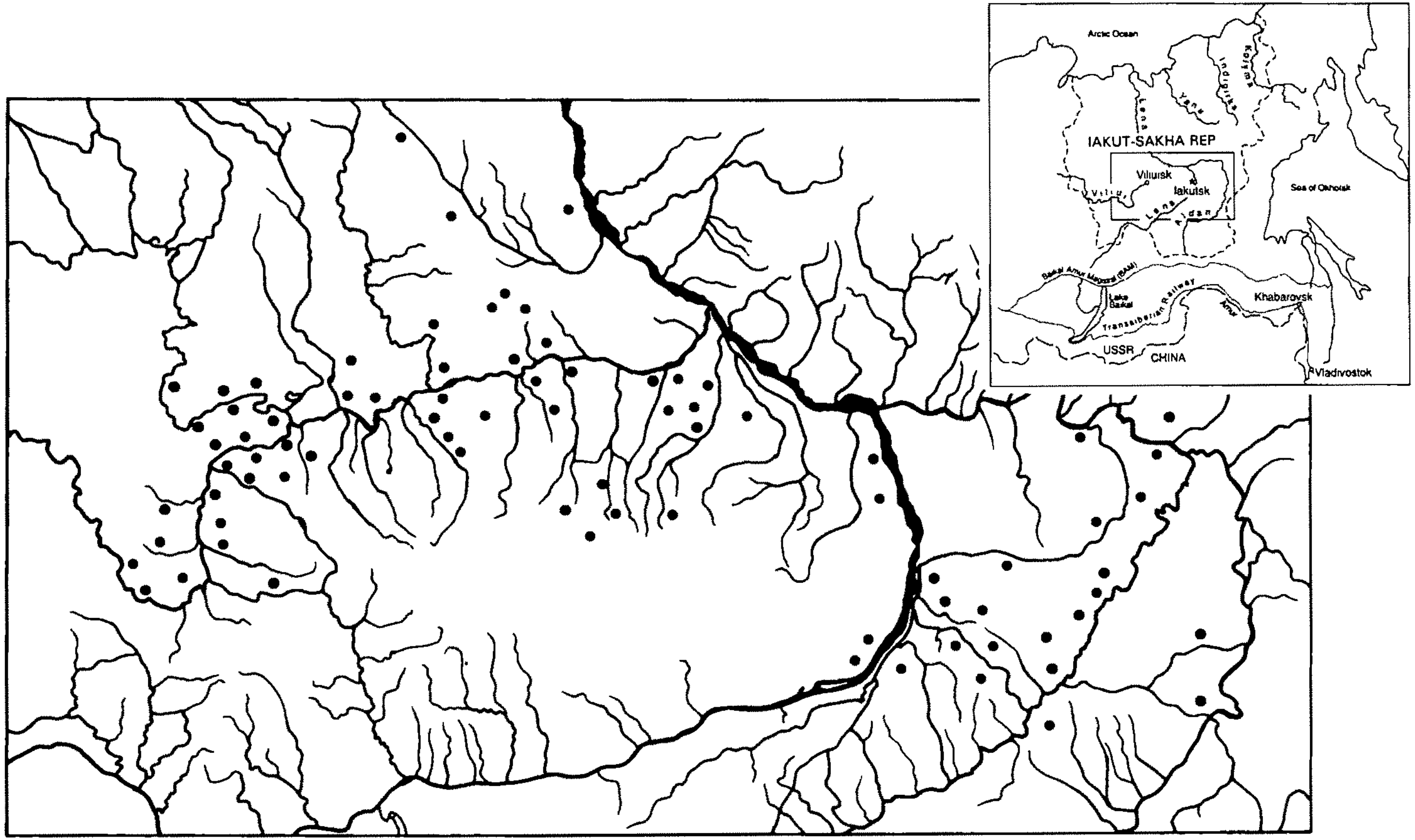

FIG. 1. Villages in Viliui, Aldan and Upper Lena regions in which VE patients have been found in 1970–1979. Inset: Sakha Republic (Iakutiia) (formerly Iakut-Sakha Soviet Socialists Republic) in Siberia.

PATIENTS AND METHODS

Patient selection and surveillance

Patients with VE were systematically identified during the decade from January 1970 to January 1980. Personnel of the Iakut Republic Health Service were trained in early VE diagnosis and encouraged to send suspected patients to the VE neurological clinic. Groups of neurologists regularly visited the disease-affected areas to examine suspected patients. Patients brought to the clinic underwent further examination, including daily neurological evaluation, consultations with a variety of specialists; and routine laboratory study of blood, urine and cerebrospinal fluid (CSF). Electroencephalography (EEG) and pneumoencephalography (PEG) were performed in almost all cases. In 1971, 1974 and 1976 selected VE patients were brought to Moscow for additional clinical, biochemical and virological studies in several institutes (Chumakov *et al.*, 1977). A final diagnosis of VE was agreed upon by a panel of qualified neurologists, based on these data, a VE Patient Registry was published (Goldfarb *et al.*, 1976). Traditional knowledge of the disease had made diagnosis of the unique syndrome by the Iakut family very accurate.

Over a period of 10 yrs, 109 newly recognized VE cases were recorded and studied (Table 1). Sixty-four patients had died before the study was completed and 27 of them were studied neuropathologically. An additional 139 living VE patients diagnosed before January 1, 1970 were also studied. The total number of VE patients covered by this study was thus 248.

TABLE 1 NUMBER OF PATIENTS WITH VE INCLUDED IN THIS STUDY

	No. of patients		
Clinical type	*New cases (registered in 1970–1980)*	*Old cases (registered before 1970)*	*Total*
Rapid (subacute)	15	0	15
Slowly progressive	49	0	49
Chronic	26	139	165
Undetermined	19	0	19
Total	109	139	248

Epidemiological procedures

All patient's residences were visited, detailed anamneses were constructed from interviews with family members and from medical records in the village clinic. Data on the patient's contacts with previously known VE patients were of special interest. Genetic study included construction of affected family pedigrees. Genealogies of the families were constructed using recollections of the senior family members and data from available registration books. Most genealogies included four to seven generations. The entire populations of nine VE-affected villages were surveyed.

Neuropathological study

Brains of five patients who had died of rapid (subacute) disease, 13 of slowly progressive disease, and nine of chronic disease, were studied histopathologically in Moscow in the late 1970s and early 1980s. No Giemsa, Levaditti or other stain for protozoa, helminths or other parasites were made. All tissue was fixed in formaldehyde. No immunocytochemical studies have yet been carried out. The published neuropathological reports are all poorly illustrated and printed on inferior-quality paper. In our current studies we plan to rectify these serious omissions.

RESULTS

Clinical features

Viliuisk encephalomyelitis is a pleomorphic disease with a variety of clinical courses ranging from relatively rapid of 2–3 mths duration to extremely prolonged of 5–6 yrs (and in rare instances more than two or three decades) duration. Most patients had a sudden febrile onset with a unique acute encephalopathy characterized by headache, bradykinesia, muscular stiffness and problems of gait followed by an intermediate phase in which symptoms disappeared or largely subsided, then a unique neurological syndrome including a return of stiffness and the appearance of a progressive dementia, and increasing severity of signs and symptoms, leading to the picture which characterizes the VE syndrome. The clinical course of VE, according to its speed of evolution, may be divided into three types of illness: (i) rapid (subacute), (ii) slowly progressive and (iii) chronic.

Rapid disease

Fifteen cases are included in this group (Table 2). Four were members of previously affected families. The first symptom was sudden onset of chills, fever and headache that quickly became excruciating. The severity of pain was aggravated by movement, light or noise. All patients had fever of 38–40°C that persisted 2–6 wks, then subsided and in some cases remained at a low-grade level for several months. Headache remained intense for several weeks. Chills, muscle and joint pains, paraesthesias with a sensation of vibration or electrical current in the extremities and pain along the nerve trunks were common. Stiffness of the neck muscles and Kernig's sign were seen in all cases. Signs of cranial nerve dysfunction, including ptosis, diplopia, strabismus, impaired convergence and accommodation were frequent findings. Bradykinesia, rigidity and dysarthria appeared between the second and fourth week after onset; hyperactive deep reflexes and spastic tetraparesis appeared at about the same time or later in the course of the disease.

All 15 patients died within 2–3 mths after disease onset, before signs and symptoms of the acute phase had subsided.

Slowly progressive disease

This group included 49 patients (Table 2) making this type of illness the most frequent among the newly diagnosed cases. It had a characteristic sequence of phases: acute, recurrent-exacerbative, fully developed, terminal.

Acute phase. There was a sudden, acute onset, as in the first group of patients, with headache, chills, fever, vomiting, confusion and somnolence followed by movement disorders with stiffness and ataxia, difficulty with speech and intellectual disorientation. The fever, headache and somnolence faded in 2–6 wks but loss of interest, apathy, poverty of movement, bradykinesis and stiffness at times persisted. Most patients, however, showed marked remission of signs and symptoms.

Recurrent-exacerbative phase. With subsidence of the fever and headache of the acute phase, some patients tried to return to work, but most could not handle it well. Behavioural changes including loss of interest, apathy and somnolence, persisted in many. After varying periods of partial remission, symptoms again exacerbated and in almost all patients recurrent headache, stiffness and weakness, and pain in the extremities reappeared. Clumsiness in fine movements and manipulations and a stiff and shuffling gait were characteristic. Many had difficulty in pronouncing polysyllabic words and exhibited unusual decomposition of speech when pressed to read or talk quickly.

Fully developed phase. The signs and symptoms of the previous phase no longer remitted and the advanced fully expressed phase of the disease set in, characterized

TABLE 2 CLINICAL AND LABORATORY FINDINGS IN 229 VE PATIENTS

	Type of disease		
	Rapid	*Slowly progressive*	*Chronic*
Number of patients	15	49	165
Age at onset, range (yrs)	18–61	14–57	17–57
Average (yrs)	40.1	35.7	35.9
Duration, range (mths) 2–13	17–73	71–420	—
Average (mths)	(6.8)	(42.5)	(194 2)
Male-to-female ratio	6 : 9	25 . 24	54 : 111
Fever	15 (100%)	38 (78%)	74 (45%)
Headache	15 (100%)	37 (76%)	57 (35%)
Coma, stupor	14 (93%)	11 (22%)	10 (6%)
Delirium	10 (67%)	6 (12%)	1 (1%)
Seizures	3 (20%)	4 (8%)	15 (9%)
Somnolence	—	24 (49%)	32 (19%)
Vomiting	11 (73%)	10 (20%)	31 (19%)
Diplopia	—	21 (43%)	49 (30%)
Meningismus	11 (73%)	6/19 (32%)	—
Apathy, slackness	12 (80%)	21 (43%)	25 (15%)
Euphoria	—	14 (29%)	98 (59%)
Memory impairment	—	31 (63%)	—
Slurred speech	—	23 (47%)	—
Incoordination	9/12 (75%)	10 (20%)	3 (2%)
Pain and weakness in extremities	—	40 (82%)	—
Obesity	—	27 (55%)	34 (21%)
Dementia	—	40 (82%)	131 (79%)
Dysarthria	—	47 (96%)	138 (84%)
Bradykinesia and rigidity	10 (67%)	39 (80%)	139 (84%)
Pyramid tract signs	15 (100%)	49 (100%)	165 (100%)
Gait disturbance	—	49 (100%)	165 (100%)
Amentia	8 (53%)	34 (69%)	—
Inability to walk	15 (100%)	31 (63%)	—
Anarthria	13 (87%)	23 (47%)	—
Lower motor neuron signs	9 (60%)	17 (35%)	—
Cerebrospinal fluid			
Cell count $\geq 7/\text{mm}^3$.	15 (100%)	43/48 (90%)	30/98 (31%)
Protein ≥ 33 mg/dl or more	13 (87%)	40/48 (83%)	17/98 (17%)
Electroencephalography			
Lowering of potential	—	47/47 (100%)	21/21 (100%)
Absence of alpharhythm	—	47/47 (100%)	21/21 (100%)
Pneumoencephalography			
Adherent meninges	5/5 (100%)	14/30 (47%)	19/46 (41%)
Hydrocephalus	5/5 (100%)	30/30 (100%)	46/46 (100%)
Brain atrophy	—	21/30 (70%)	42/46 (91%)
Neuropathology			
Necrosis-inflammation	5/5 (100%)	13/13 (100%)	—
Spongiosis and gliosis	—	13/13 (100%)	9/9 (100%)

by stiffness, and even rigidity, after which the disease receives its traditional Iakut name, 'bokhoror'. Bradykinesis and impoverished movement became more apparent, generalized stiffness and ataxia persisted and progressed in severity. There was difficulty initiating voluntary movements and patients bent forward markedly to initiate walking. Almost all patients had a marked dysarthria, with speech becoming progressively slow, thick, slurred, muffled, arrhythmic and less distinct. Speech disturbance, when less apparent, could be demonstrated by urging the patient to speak or read more rapidly. Discordant contractions of the participating muscles were an evident cause of the dysarthria. Many patients also had signs of dysphasia, word amnesia and impaired comprehension of speech. A global dementia slowly developed.

Pyramidal tract involvement was observed in all VE patients, manifested by hyperactive deep reflexes, clonus and positive Babinski sign. Noticeable was the propensity to bend forward to initiate walking. The gait had evident signs of stiffness and spasticity, which steadily progressed until the patient was unable to walk. Disintegration of skilled movements and apraxia were also notable. Sphincteric incontinence was present in most of the cases. Seventeen patients had overt signs of lower motor neuron involvement. Petrov (1970, 1973, 1980, 1987) emphasized the existence of a separate amyotrophic subtype of VE. Signs of cerebellar dysfunction were found in only 10 patients.

Generalized convulsive seizures were observed in six patients, in five of whom it first occurred during the acute phase. Two had recurrent seizures and none showed typical epileptic EEG activity.

Some patients suffered hypothalamic involvement: five had typical autonomic paroxysms of adrenal or vago-insular type; many became obese in the early phase of their disease, and some later showed cachexia. Sexual dysfunction, especially impotence, was noted in the majority of male patients.

Terminal phase. In the late phase of the disease, patients became yet more profoundly disabled. They were mute, were unable to communicate or walk, exhibited global dementia and were usually incontinent. Contractures and decubitus ulcers appeared if nursing care was not meticulous. Death occurred from hypostatic pneumonia or renal insufficiency secondary to sphincteric problems.

Chronic disease

This group included 165 patients who had an extremely prolonged course of disease (Table 2). Many had no acute phase recorded or the acute phase was mild. The clinical characteristics of the chronic disease were very similar to those of the slowly progressive type, but most had stabilized in advanced disease and showed no further progression. Death occurred secondary to accidents or other diseases, and some lived to be very old. Many patients lived in their homes, and those who required extensive nursing were admitted to the Home for Chronic VE Patients in Viliuisk.

Laboratory findings

Although numerous laboratory and functional tests have been performed in VE patients, only early testing of the CSF and PEG were useful for the diagnosis. In patients with *rapid disease*, CSF showed inflammatory changes with moderately elevated protein concentration and a pleocytosis of between 20 and 90 cells/mm^3. Most of the cells were mononuclear. Pneumoencephalography revealed signs of an adherent process in the meninges and high pressure hydrocephalus. In *slowly progressive* disease, increased intraventricular pressure was noted in seven of 14 patients examined (Gogolev, 1974). The number of cells in CSF ranged from seven to 60/mm^3. Pleocytosis persisted for many years through all the phases, declining slowly after the sixth year of illness. The protein concentration was moderately elevated.

In chronic disease, CSF did not show any abnornal changes. Pneumoencephalography performed in 25 patients in late disease showed communicating hydrocephalus with a considerable widening of the lateral and third ventricles (Baranov, 1972). High-pressure hydrocephalus seen in the early stages was due to adherent leptomeningitis with subarachnoid obstruction. Later in the course of the disease, diffuse brain atrophy and atrophic low-pressure hydrocephalus developed. Electroencephalographic recordings revealed a diffuse lowering of electric potentials, mainly in the frontal and parietal regions. Alpha rhythm was replaced by low-amplitude, irregular beta rhythm with superimposed polymorphic slow waves (Moshanova, 1972).

Pathological findings

No neuropathology on VE cases has been done in the past decade. We base our description on the published reports of the 27 autopsies performed before this study was completed and reported in the early 1970s and 1980s. No electronmicroscopic studies have been performed and brain specimens have not yet been fixed or stained adequately for protozoa and other parasites, nor have frozen brain specimens been properly collected for *in situ* hybridization or immunohistochemical studies.

Rapid disease

Five cases were studied. The meninges were thickened and cloudy and microscopically showed widening of subarachnoid spaces, infiltration with mononuclear cells and, to a lesser extent, with plasma and polymorphonuclear cells. Vascular cuffs consisting mainly of mononuclear cells were widespread. In the cerebral hemispheres, multifocal areas of necrosis were seen in all the examined fields of the frontal, temporal and occipital cortex. Each area consisted of an eosinophilic necrotic mass surrounded by mononuclear and microglial cells in the inner and reactive astrocytes in the outer layer ('fresh necrotic focus') (Savinov *et al.*, 1972). Another type of necrotic lesion had a necrotic centre filled with condensed granular material and was surrounded by macrophages and microglial cells, the outer layer being composed of a more sclerotic coat of tightly packed astrocytes ('late necrotic focus') (Savinov *et al.*, 1972). There was a complete destruction of all nerve cells within the necrotic foci. The cortex was rarefied and diffusely infiltrated by macrophages and microglial cells. Perivascular cuffs containing mononuclear cells were frequent in affected cortical areas. The white matter was not severely affected, with only occasional perivascular inflammatory reactions.

Multifocal necrosis and inflammatory changes in the basal ganglia were very similar to that described in the cerebral cortex. 'Late necrotic foci' were prevalent in brain stem nuclei located in the tectum, basis pontis and medulla, which showed extensive neuronal loss and marked inflammatory cell infiltration. In some areas, there were cavities within the rarefied zones. Areas of necrosis were present in all cerebellar layers: granular, Purkinje cells and molecular. There was a complete destruction of neurons, including Purkinje cells, inside and outside of necrotic areas. The inflammatory changes were of the same character as seen in the cerebral cortex. Necrotic foci and some vacuolated neurons were found in inferior olives, substantia nigra and reticular formation. Chromatolysis and accumulation of lipofuscin were seen in surviving neurons.

In the spinal cord, fresh necrotic foci were found in the anterior and posterior horns and in the lateral grey matter. Perivascular cuffs were plentiful in the affected areas. In the white matter, swollen fragmented axons and a loss of myelin staining were often seen. Rarefaction of the nerve fibrils was prominent, especially in the thoracic and lumbar areas of the corticospinal tract.

Slowly progressive disease

Thirteen cases were studied. The meninges were thickened, cloudy, fibrosed and infiltrated by mononuclear and plasma cells. Perivascular cuffs were very pronounced. Within the cerebral hemispheres, both fresh and late necrotic lesions were seen together (Savinov *et al.*, 1972; Avtsyn *et al.*, 1983). The proportion of fresh to late lesions varied from patient to patient. Gliosis was noted around some of these areas. Perivascular cuffs were more frequently observed in areas adjacent to the fresh necrotic foci.

Multiple fresh and late necrotic foci were found in basal ganglia, substantia nigra, reticular formation, oculomotor nuclei and other grey matter formations. Moderate perivascular cuffing was present, but was uncommon. Gliosis was quite evident in the inferior olives. Within the pyramids, changes consisted of mild swelling of the myelin sheaths and loss of axis cylinders. Similar changes were found throughout the corticospinal tract and posterior columns.

Chronic disease

Nine cases were studied. The meninges were fibrotic, the brain was small and atrophic, the ventricles were markedly enlarged. Microscopically, the cerebral cortex was atrophic in various areas of the brain; severe gliofibrosis was present everywhere, and areas of spongiform change were frequent in the thalamic and hypothalamic areas. Cystic atrophy of the basal ganglia was very prominent. Spongiform state and severe gliosis were the main features in the mid-brain and pontine nuclei, and in the spinal cord grey matter. Loss of axis cylinders in the pyramids and other long tracts of the spinal cord was severe. More recent necrotic lesions were sometimes seen in various places against a background of old fibrotic changes. Perivascular cuffings were not usually present in the chronically affected areas in patients with a 6-yr or longer history of disease.

Epidemiology

Geographical distribution

In previous decades, most of the recorded cases came from the Middle Viliui area (Fig. 1 and Table 3), where VE was first recognized. Diffusion of VE from the Middle Viliui to the adjacent Upper and Lower Viliui areas became noticeable in the 1950s, after migration considerably increased; and more recently the disease has penetrated into the highly populated Lena-Aldan Valley. Long-time physicians in the Lena-Aldan area regarded VE as a new disease, transmitted from people who came from the Viliui Valley during World War II and in the post-war period (Goldfarb *et al.*, 1972*a*,*b*, 1975). Data indicate a 17% shift in the number of newly detected VE cases from the Middle Viliui to other areas over the last decade (Table 3).

All the registered VE patients were born and most of them lived their entire lives in small villages. Most were Iakuts, seven were Tungus, and 11 were born from Iakut-Tungus mixed marriages.

TABLE 3 PLACES OF BIRTH OF VE PATIENTS REGISTERED IN 1940–1969 AND IN 1970–1979

	Year of registration	
	1940–1969	*1970–1979*
Area	*No. of patients (%)*	*No of patients (%)*
Middle Viliui	67 (48)	34 (31)
Other regions of the Viliui Valley	45 (32)	40 (37)
Lena and Aldan Valleys	27 (20)	35 (32)
Total	139 (100)	109 (100)

Year and month of onset

During the 1970s, 10–16 individuals contracted the disease each year. The greatest number of VE cases have started in May, June or July (50 of 84 with a known month of onset), which may be due to more intensive outdoor activities such as hunting, fishing and pasturing of cattle that subject people to frequent exposure to possible environmental factors provoking the onset of the disease.

Age at onset

The youngest of the patients was 14 yrs, and the oldest was 61 yrs. Most patients were in the age range 30–50 yrs, with an average age at onset of 35.3 yrs for men and 39.8 yrs for women.

Duration of illness

The mean duration, estimated by determining the 50% mortality point on the duration-survival curve, was 3.4 yrs for men and 3.9 yrs for women.

Incidence, prevalence and mortality rates

The incidence and mortality rates were calculated as average annual indexes over a period of 10 yrs, January 1, 1970 to January 1, 1980, and the point prevalence rate was estimated for the middle of this interval, January 1, 1976. The highest incidence, prevalence and mortality rates were found in the Middle Viliui area and the lowest in the Lena-Aldan Valleys. Nearly one per 1000 of the adult Iakut population has died of VE each year in the Middle Viliui area, the average age at death being 42.6 yrs for men and 48.9 yrs for women, which is 20 yrs younger than the current life span of the Iakut population.

Family clustering

Complete family histories were obtained from 194 affected families. In 27 of them multiple VE cases have been recorded, two per family in 24 and three per family in three. Types of relationship between VE patients belonging to the same family were: full siblings in eight families, full siblings and a niece in one, full siblings and an adopted genetically unrelated brother in one, half-siblings in one, parent and child in three, cousins in one, spouses in two, parent and adopted child in one, a family member and adopted brother or sister in five, and unrelated family members (in-laws) in four families.

Iakut families are usually large and have a complex structure. Out of 1090 families studied in six Middle Viliui villages, 26 (2.5%) consisted of 10 or more members, and

331 (30%) of five or more members. About 10% of the families have adopted genetically unrelated children. Related families living in the same household and adoption are very old traditions providing additional chances for survival in the severe life conditions (Goldfarb *et al.*, 1979*a*).

With large families, there is always a possibility that clustering of cases happens simply by chance. From the actual sizes of the 1090 studied families and VE prevalence rate in the area (16.4 per 1000), we calculated the statistical expectation of a number of VE cases per family (Table 4). The calculated distribution differed from the observed by a factor $\chi^2 = 18.4$, indicating that multiple cases of VE were observed more frequently than could be explained by chance alone. This applies to genetic kin as well as non-genetic relatives; their separate χ^2 estimates were 9.4 and 6.8, respectively.

TABLE 4 EXPECTED AND OBSERVED NUMBER OF IAKUT FAMILIES WITH 0, 1, 2 OR 3 VE PATIENTS PER FAMILY IN SIX VILLAGES OF THE MIDDLE VILIUI AREA

	Total no. of families	*No. of families with VE cases*				
		0	*1*	*2*	*3*	χ^2
Expected	1090	1028.93	59 06	1 95	0 05	—
Observed	1090	1041	35	13	1	18.4

Genetic studies

Segregation analysis

The strong familial pattern of VE occurrence made some investigators believe that VE is a genetic disease with an autosomal recessive pattern of inheritance (Zubri *et al.*, 1975, 1977), but a careful analysis based on a larger number of families did not confirm this theory. Using segregation analysis technique published by Lejeune (1958) we found very low coefficients of inheritance in the affected families from the Middle Viliui area ($P = 0.073 \pm 0.021$), as well as in the families from the other regions of the Viliui Valley ($P = 0.034 \pm 0.022$), and $P = 0$ in the families from Lena-Aldan Valleys (Table 5); these data are not compatible with an autosomal recessive mode of inheritance. A separate calculation for sibships in which the patients and their siblings have already reached age 50 yrs also negates the autosomal-recessive hypothesis ($P = 0.025 \pm 0.031$).

Hereditary predisposition to the disease

Familial predisposition to VE was studied using Falconer's methodology modified by Edwards (1969). The coefficient of heritability $H = 22.14\%$ for the Middle Viliui area and $H = 28.94\%$ for the other Viliui Valley regions (Table 6) indicate that heredity has some role in the VE aetiology.

Coefficients of relationship and inbreeding in the affected kindreds

A suggestion has been made about a higher hereditary predisposition to VE of the Iakuts belonging to certain clans (Umanskii, 1974). Because the clan distinction of the Iakuts has been lost, we calculated coefficients of relationships: (i) between the affected (P) families P×P; (ii) between the affected and non-affected (N) families P×N; and (iii) between the non-affected families N×N, for the population of three villages in

TABLE 5 SEGREGATION ANALYSIS IN 163 VE-AFFECTED FAMILIES*

Area	*Sibship*	*No. of families*	*Total*	*VE patients*
Middle Viliui	2	18	36	18
	3	20	60	21
	4	14	52	17
	5	12	60	14
	6	6	36	7
	7	5	35	5
	8	2	16	2
	9	1	9	2
Total		78		86
			$P = 0.0731 \pm 0.0217$	
Other regions of the Viliui Valley	2	11	22	11
	3	11	33	11
	4	12	48	13
	5	6	30	6
	6	3	18	4
	8	1	8	1
Total		44		46
			$P = 0.0340 \pm 0.0228$	
Lena and Aldan Valleys	2	5	10	5
	3	12	36	12
	4	6	24	6
	5	10	50	10
	6	3	18	3
	7	2	14	2
	8	2	16	2
	10	1	10	1
Total		41		41
			$P = 0$	

the Middle Viliui area (Table 7). The affected families appeared to be genetically closer in Borogon, one of the three examined villages. This may be an indication that some of the clans are indeed more predisposed to the disease than others (Goldfarb *et al.*, 1979*b*).

Inbreeding coefficients were also calculated separately for affected and non-affected families. The values were very low in all the studied populations: 0–0.00023, no difference between the affected and non-affected families. The disease is clearly not caused by a single mutation.

Epidemiological studies indicating communicability

Common exposure. Schematic data in Fig. 2 show time of common exposure in relation to the disease onset in members of the same family. In 27 families a household member (some of them genetically unrelated family members) contracted the disease after a prolonged intrafamily contact with an already ill VE patient. In nine other families, the affected members lived together before the clinical disease started. These observations suggest that horizontal disease transmission may be occurring between family members.

In Table 8, duration of common exposure (time *BC*) and estimation of the incubation

TABLE 6 HERITABILITY (H%) IN VE-AFFECTED FAMILIES*

Area	Group	Total no	No of VE cases	Prevalence rate (%)	H%
Middle Viliui	Unaffected families	4762	64	1.34	—
	Members of affected families				
	Parents, siblings, children	498	13	2.61	22.1
	Uncles, aunts, nephews, nieces	215	4	1.86	20.8
	Cousins	279	4	1.43	8.0
Other regions of the Viliui Valley	Unaffected families	31 955	58	0.18	—
	Members of affected families				
	Parents, siblings, children	299	2	0.66	28.9
	Uncles, aunts, nephews, nieces	234	1	0.42	36.6
	Cousins	225	0	0	0

*Individuals of 20 yrs or older are included.

TABLE 7. COEFFICIENTS OF RELATIONSHIP BETWEEN THE VE-AFFECTED FAMILIES

Village	Relationships between families*	No. of cross relationships	Coefficients summarized	Average coefficient of relationship
Bagadja	P×P	36	0.0147	0.0004
	P×N	280	0.4764	0.0017
	N×N	587	1.2489	0.0021
	All families	903	1.74	0.0019
Borogon	P×P	45	0.4525	0.0101
	P×N	560	2.5305	0.0045
	N×N	1540	9.2415	0.006
	All families	2145	12.22	0.0057
Teia	P×P	28	0.3028	0.0108
	P×N	488	2.672	0.0055
	N×N	1830	14.48	0.0079
	All families	2346	17.46	0.0074

*P = affected; N = unaffected

period were made using methodology published by Poskanzer (1965). The average common exposure was 9.7 yrs, 14.1 yrs for genetically related and 4.6 yrs for adopted family members. Minimal common exposure was 1 yr. In 22 cases out of 30 the common exposure occurred during childhood and/or adolescence. The average incubation period was 15.7 yrs, 17 yrs for genetically related patients, and 11.3 yrs for genetically unrelated family members.

Migration and VE diffusion. Among 205 adults who immigrated into the high risk Middle Viliui area, three individuals or 14.6 per 1000, contracted VE, which is close to the prevalence rate in the high-risk area (16.4 per 1000) into which they had immigrated and different from that in areas of their birth (less than 1 per 1000). Conversely, the estimated prevalence rate among those leaving the high-risk area fell from 16.4 to 6.7 per 1000, but still was higher than in the areas into which they immigrated.

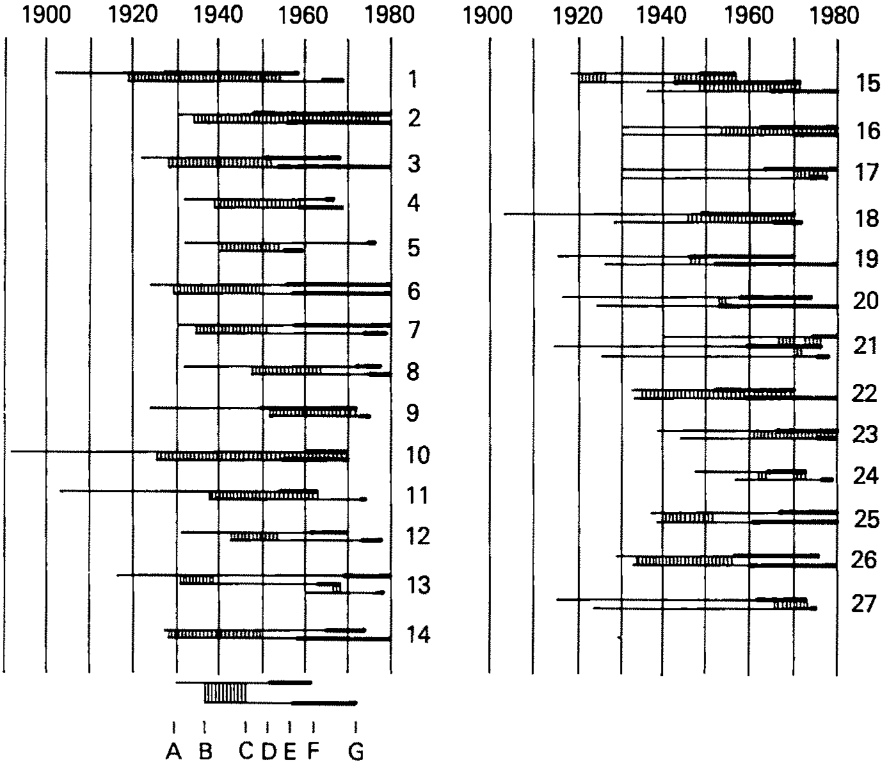

FIG. 2. Duration of common exposure (vertical hatching) and the incubation time in patients coming from families with multiple VE cases. The patients were full siblings in families 1–8, parent and son or daughter in families 9, 10 and 11; half-siblings in family 12; two siblings and their niece in family 13; cousins living together in family 14; two siblings and an adopted individual in family 15; spouses in families 16 and 17; adopted non-blood relatives in families 18–27. In the last group, families 15, 22, 23, 24 and 25 adopted their children as infants or in early childhood. Denominations· A = year of birth of the first affected person; B = year of birth of the second affected person; C = year of termination of the common exposure; D = year of onset of VE in the first affected person; E = same in the second case, F = year of death in the first affected person, G = same in the second case.

During the 10-yr study we were able to follow the progress of VE penetration into the densely populated Lena-Aldan Valleys. Cases of VE were found in 33 of 130 Lena-Aldan villages. Four of these 33 villages had three or more VE cases, which cannot be explained by chance alone. The sequence of events in these four Lena-Aldan villages was as follows. The first victims in Kyiy, a village in the Lena-Aldan valley, were two migrants from the high-risk area. One of them arrived already ill with VE, the other became ill 4 yrs after immigration. More than two decades later, a young native of this village who had never visited the Middle Viliui area contracted the disease. In Keptin, a previously VE-free village, an immigrant from the high-risk area arrived shortly after the onset of the disease. A younger member of a family that adopted this first patient fell ill with VE 14 yrs later, and in several years a village native who had close intrahousehold contact with the second patient also developed VE. During the next decade, two other lifelong village residents contracted VE. In Lomtuka, the first recognized

TABLE 8 DURATION OF A COMMON EXPOSURE AND INCUBATION PERIOD IN FAMILIES WITH MULTIPLE VE CASES

Families with genetically related VE patients	*BC*	*BD+CD+BE+CE/4*
1	8	15.75
2	14	8.75
3	23	12.75
4	20	13.5
5	15	18.5
6	21	16.5
7	17	22.5
8	16	17.5
9	0	5.5
10	29	15.75
11	16	15.25
12 (1)	5	16.0
19	11	18.5
20 (1)	8	30.0
20 (2)	0	5.25
26	22	22.5
Average	14.1	*I* = 17.0

Families with genetically unrelated (adopted) VE patients	*BC*	*BD+CD+BE+CE/4*
12 (2)	0	4.25
13	9	6.5
14	0	1.0
15	3	7.75
16	0	2.75
17	0	2.25
18 (1)	0	2.0
18 (2)	0	3.25
21	15	9.75
22	4	3.5
23	1	2.25
24	11	17.5
25	22	13.5
27	0	2.5
Average	4.6	*I* = 11.3

VE patient was a migrant from Middle Viliui, and three subsequent cases appeared among native villagers who had never visited the high-risk area. In Salban, the first VE case occurred in a migrant from Viliui Valley. Sixteen years later, another Viliui immigrant and a native villager developed VE.

These data indicate that VE foci in formerly unaffected villages originate from immigrants coming from the high-risk area and are followed some time later by cases among the indigenous population living for years in close contact with the immigrated VE patients.

DISCUSSION

The cause of VE is still unknown. Inoculation of VE blood, serum, CSF and brain tissue into tissue cultures has failed to yield a causative agent. Inoculation of these tissues into laboratory animals, including monkeys, by the intracerebral and intraperitoneal routes has not produced any disease in the recipients. In one series of such inoculated mice, however, a fatal encephalitis developed. This virus was called the VE virus. In the 1950s, Casals (1963, 1965) showed that the VE virus was related to mouse encephalomyelitis (MEM) virus GDYII strain, and less closely to encephalomyocarditis (EMC) virus. Patients with VE develop no antibody to this virus. Viruses of the MEM group are commonly isolated when working with laboratory or wild mice (Brody, 1965).

More recently, another candidate agent has been isolated from the autopsied brain of a woman (KPN) with chronic VE by inoculating *in vitro* cultivated MIO rhesus monkey cells and DKCh (dog kidney) cells (Sarmanova *et al.*, 1975). The agent, designated KPN virus, was first detected by indirect immunofluorescence using VE patients' sera and, after five passages, a cytopathic effect developed. It was sensitive to lipid solvents (ether, chloroform), sodium deoxycholate and formaldehyde (Karavanov *et al.*, 1975, 1981). Suckling mice, rats, hamsters and adult mice develop an acute encephalitis in 4–7 d after intracerebral inoculation with the KPN virus, but serial passage of the disease in these laboratory animals has been unsuccessful. The agent caused low-grade encephalitis after a long incubation period in rhesus monkeys. Its low titre in tissue culture and infected animal brain has made its characterization difficult. Further attempts to obtain this virus from 13 biopsy and two autopsy brain specimens of VE patients have been unsuccessful. Patients with VE fail to develop neutralizing antibody to this agent and its possible aetiological role in VE is uncertain (Gajdusek, 1982; Gajdusek and Goldfarb, 1991). Chumakov and Karavanov (1986) have demonstrated that the KPN agent has been mistakenly thought to be a virus. It has a 5S-RNA which sequences identically to that of *Acanthamoeba castellanii*. Whether this free-living amoeba has been a chance contaminant or has, in fact, come from a VE patient's brain has not been proved.

The Iakut area lies north of the belt of tick-borne Russian spring-summer encephalomyelitis and of hantavirus-caused haemorrhagic fever with a renal syndrome. Our extensive serological study of sera from more than two dozen VE patients has failed to suggest that the causative agent may be related to any of the microbial agents known to produce central nervous system infections in temperate or arctic regions.

CONCLUSION

Viliuisk encephalomyelitis is a unique, progressive and fatal neurological disorder. At present, about 10–16 new cases of the disease appear each year. Some patients die during the acute meningoencephalitic phase of the disease, but the majority undergo a slowly progressive course and eventually die 2–6 yrs after onset. Some chronic VE patients survive for decades.

Half of the registered patients have an acute phase of the disease with chills, fever and headache, influenza-like muscle pains, stiffness, ataxia, extreme lethargy and cranial nerve dysfunction, including ophthalmoplegia. There is usually a CSF pleocytosis and a moderately elevated protein concentration. The acute phase lasts several weeks to months and in some cases leads to early death. We observed 15 such cases, five of which were examined neuropathologically. Focal micronecrosis accompanied by perivascular cuffings were seen mostly in the grey matter. The pathological changes were characteristic and distinct and clearly established VE as a unique disease. The slowly progressive type of VE syndrome, often developing after a long or short remission from the acute phase appears with bradykinesis, stiffness, ataxia, dysarthria, dementia and neurological signs of spastic parapareses and is fatal within 2–6 yrs after onset. Neuropathologically, it is characterized by primary diffuse multiple fibrotic and micronecrotic foci with marked inflammatory reactions in the grey matter, perivascular cuffing with leucocytes, infiltrated and fibrosed meninges. Some regions of spongiform change are found in slowly progressive and chronic forms of this disease. The fibrotic meninges and adhesions of the cerebral cortex to meninges impede the CSF circulation and lead to an increased intraventricular pressure that in time produces hydrocephalus and brain atrophy. The neuropathology suggests chronic infection provoking extensive inflammatory response.

Viliuisk encephalomyelitis is a frequent disease in the Middle Viliui area, where the number of cases is accumulating to a prevalence rate of over 1%, and the death-rate ranks the disease among the five most important causes of death in the area. In recent years, the disease has been slowly disseminated to adjacent densely populated regions following paths of migration of affected Iakut populations. The immigrants introduce this disease to non-immigrant genetically unrelated Iakut, who have lived for prolonged periods in households of VE patients.

The clinical, pathological, epidemiological findings point to an infectious aetiology, but preliminary studies in Iakutia, including animal and tissue culture inoculations of brain tissue, CSF and blood from patients, have yet failed to demonstrate an aetiologic agent.

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OCCURRENCE OF A MULTIPLE SCLEROSIS-LIKE ILLNESS IN WOMEN WHO HAVE A LEBER'S HEREDITARY OPTIC NEUROPATHY MITOCHONDRIAL DNA MUTATION

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SUMMARY

Eight women are described who presented with bilateral, usually sequential, optic neuropathy, six of whom later developed a neurological syndrome indistinguishable from multiple sclerosis (MS). Magnetic resonance imaging, performed in five of the patients with an MS-like illness and in the two others with optic neuropathy alone, showed widespread white matter lesions as seen in MS. All of these women had matrilineal relatives with Leber's hereditary optic neuropathy, although this was not always apparent at presentation, and the most common mitochondrial DNA mutation associated with this disorder was detected in each of the women and their affected relatives. On the basis of observations made in these patients, the clinical features of Leber's hereditary optic neuropathy in males, and evidence for mitochondrially encoded peptides involved in the immune response in rodents, we propose that optic nerve damage in this disease could be immunologically mediated and that mitochondrial genes may contribute to susceptibility to MS.

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) characteristically causes severe visual loss in otherwise healthy young men. At onset the optic discs often appear swollen with tortuous retinal arterioles and peripapillary telangiectases, and optic atrophy supervenes within 2 mths. Loss of visual acuity is generally severe (6/60 or less) and persistent. Leber's hereditary optic neuropathy is exclusively maternally transmitted; in contrast to X-linked diseases, the descendants of male patients are not affected. This pattern of inheritance is consistent with an underlying mutation of mitochondrial DNA (mtDNA), and three such mutations, exclusively found in affected families, have been described (Wallace *et al.*, 1988; Howell *et al.*, 1991*a*; Huoponen *et al.*, 1991). The commonest is a point mutation at position 11778 of mtDNA which results in an amino acid change in a mitochondrial respiratory chain complex I subunit, NADH dehydrogenase 4 (ND4) and, coincidentally but usefully, loss of a recognition site for the restriction endonuclease *Sfa*NI (Wallace *et al.*, 1988). The presence of this mutation is associated with a poor prognosis for recovery of vision (Holt *et al.*, 1989; Newman *et al.*, 1991). In many

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patients, and particularly their asymptomatic relatives, a mixture of mutant and normal mtDNA is present in leukocytes (heteroplasmy; Holt *et al.*, 1989).

An association between LHON and multiple sclerosis (MS) was suggested long ago (Lees *et al.*, 1964), but this was difficult to substantiate prior to the availability of modern investigative techniques. We have recently studied six females with a family history suggesting LHON, who presented with bilateral optic neuropathy but later developed other neurological features compatible with a diagnosis of MS. This diagnosis was supported by investigations including magnetic resonance imaging (MRI). A further two females with LHON in the absence of other neurological symptoms had the characteristic features of MS on MRI. All had the 11778 base pair (bp) mtDNA mutation.

PATIENTS AND METHODS

Methods

Deoxyribonucleic acid (DNA) was extracted from 20 ml blood from each patient using standard techniques and analysed for the 11778 bp mutation as described previously (Holt *et al.*, 1989). Samples (1 μg) of DNA were digested with up to 5 units of *Sfa*NI under conditions recommended by the manufacturers (New England Biolabs, USA) with the addition of bovine serum albumin and spermidine. Fragments of DNA were separated by horizontal agarose gel (1.7%) electrophoresis for 16 h at 34 V and then transferred to nylon membrane (Hybond-N, Amersham, UK) by Southern blotting. A fragment of Hela cell mtDNA (11680–12570 bp), cloned in M13, was labelled with ^{32}P by the random primer method, prior to hybridization to the filters under conditions recommended by Amersham. Fragments were visualized by autoradiography for 8 h to 10 d at −70°C. The relative proportions of the 1.6 kb and 0.7 kb mtDNA fragments were assessed using an LKB Ultroscan densitometer.

For HLA genotyping, DNA was digested with *Taq*I according to the manufacturers' (Northumbria Biologicals Ltd, UK; NBL) instructions and electrophoresed in 0.7% agarose gels for 20 h at 30 V prior to Southern blotting onto Hybond-N. The filters were hybridized sequentially to the following HLA class II region complementary DNA fragment probes: DRB, pRTV1 (Bidwell and Jarrold, 1986), a 517 bp insert cut from pSP64 with *Pst*I (NBL); DQA, pDCH1 (Auffray *et al.*, 1984), a 797 bp *Pst*I excised insert from pBR322; and DQB, pII-B-1 (Larhammar *et al.*, 1982), a 620 bp insert restricted from pBR322 by *Ava*I (NBL). Probes were labelled by the random primer method and hybridized to filters in the presence of denatured salmon sperm and 0.25% non-fat dried milk. Autoradiographs were exposed for 24–72 h at −70°C.

Magnetic resonance imaging was performed on a 0.5T Picker superconducting scanner. The whole brain was imaged using a T_2-weighted sequence ($SE_{2000/60}$) with 5 mm contiguous axial slices. The optic nerves were imaged using a binocular surface coil, a short inversion time inversion recovery sequence ($IR_{2000/40/150}$), and 5 mm contiguous coronal slices from the posterior globe to the chiasm.

Case reports

Case 1. This 40-yr-old woman was well until the age of 26 yrs when she noticed visual loss in the left eye, preceded by retrobulbar pain for 3 d. Three months later the right eye was similarly affected, again associated with pain. At this time she was noted to have a hyperaemic optic disc on the right with a small haemorrhage and some retinal oedema. On examination 2 wks later visual acuity was counting fingers (CF) bilaterally. There was a large central scotoma on the right and an upper altitudinal defect on the left. The left disc was pale and slightly swollen and the right fundus showed hyperaemia around the disc. Computerized tomographic (CT) scan of the brain and orbits was normal. Visual evoked potentials (VEPs) to pattern reversal were undetectable. Cerebrospinal fluid (CSF) was normal, apart from showing a paretic Lange curve and slightly increased immunoglobulin (Ig) concentrations; the pattern was not oligoclonal.

She was diagnosed as having bilateral optic neuritis and treated with intramuscular ACTH but her vision never improved. Six months later she had paraesthesiae in the hands and below the waist for a few weeks, without any abnormal findings on examination. After a further 2 yrs she had an episode of loss of sensation in her feet and subsequently transient perineal sensory disturbance. Since 1982 she has had occasional sensory symptoms in the limbs which have largely remitted but she now has persistent tingling and slight weakness of the left hand and some urgency of micturition. She has experienced Lhermitte's symptom from time to time.

There was no family history of neurological or ophthalmological disease at the time of presentation, but in 1988 her younger brother developed bilateral sequential optic neuropathy at the age of 31 and was diagnosed as having LHON. The patient's mother was an orphan and nothing was known about her family.

On examination in 1991 she had acuities of 1/60 right, 6/60 left, and bilateral optic atrophy with large central scotomas. Power and coordination were normal in the limbs but she had brisk reflexes and extensor plantar responses. Vibration sense was absent to the ribs and pin prick appreciation reduced below the left elbow. T_2-weighted brain MRI showed multiple periventricular and subcortical white matter lesions in the cerebral hemispheres (Fig. 1), as well as intrinsic brainstem and cerebellar lesions (Fig. 2), all characteristic of MS. Optic nerve imaging showed small nerves bilaterally with increased signal throughout their length.

Case 2. This 46-yr-old woman developed generalized tonic-clonic and occasional absence seizures at the age of 16 yrs; these had persisted on treatment with carbamazepine (600 mg/day) and occurred approximately every 3 mths. When aged 25 yrs, shortly after the birth of her third child, she developed visual loss, first involving the left eye but affecting the right eye after a few weeks. Her visual failure has never recovered. In 1988 she became aware of progressive clumsiness, initially affecting the left arm and leg, associated with urgency of micturition. In 1990 she noticed weakness and numbness of the left hand. Her brother had been investigated for blindness during his twenties, but no further details of this are available.

On examination in 1991, visual acuities were perception of light (PL) on the right and hand movements (HM) on the left. Visual fields were difficult to assess, but appeared constricted on the left to a large white target. The pupillary reflexes to light were slow and there was bilateral optic atrophy. There was a mild slurring dysarthria and generalized limb ataxia with normal power and reflexes but extensor plantar responses. Vibration sense was impaired below the iliac crests. She had a broad based ataxic gait with Rombergism.

A CT scan showed generalized atrophy, most marked in the cerebellum and brainstem. Plasma carbamazepine concentrations were in the therapeutic range. Pattern-reversal VEPs were absent; flash evoked occipital responses were degraded and variable. Electroretinograms were normal. Somatosensory evoked

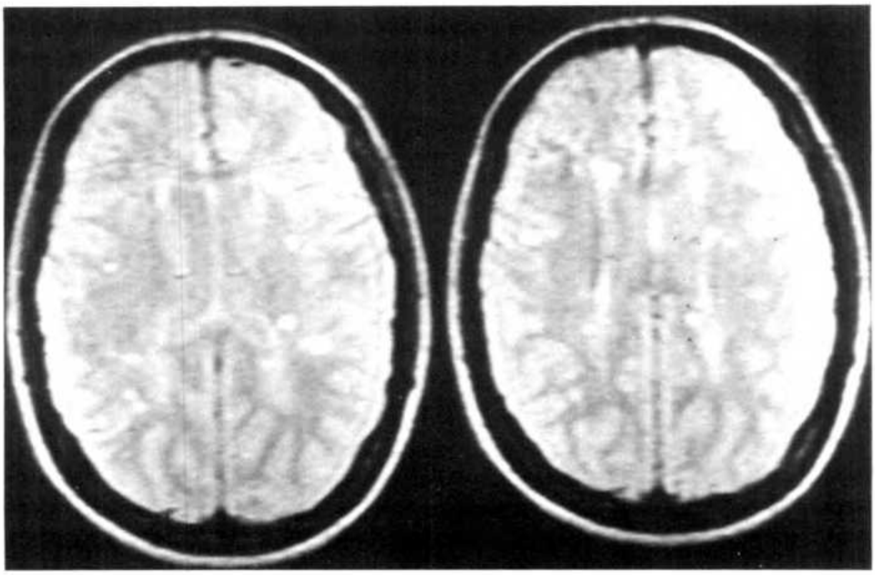

FIG. 1. *Case 1.* T_2-weighted MRI showing multiple cerebral white matter lesions characteristic of MS.

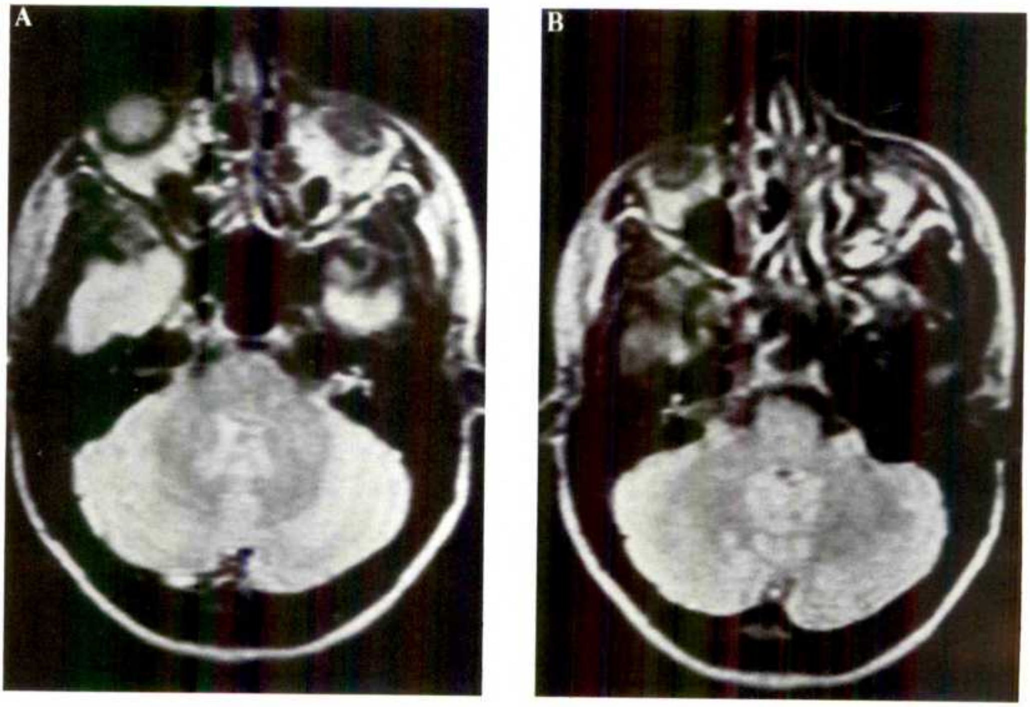

FIG. 2. *Case 1*. T_2-weighted MRI showing lesions in (A) the floor of the fourth ventricle and (B) the cerebellar white matter, consistent with MS.

potentials to median nerve stimulation were undetectable over the cervical cord and grossly delayed at the cortex (35.1 ms and 31.8 ms). Brainstem auditory evoked potentials and nerve conduction studies were normal. An electroencephalogram showed frequent bilateral frontotemporal spike and wave discharges. The CSF contained oligoclonal immunoglobulin on two occasions (in 1987 and 1989). T_2 weighted brain MRI showed multiple periventricular and discrete cerebral white matter lesions typical of MS. In addition, there was marked supra- and infratentorial atrophy and symmetrically increased signal in both pyramidal tracts in the medulla and the medial cerebellar grey matter.

Case 3. This 39-yr-old female (III.23, Fig. 3) presented at the age of 28 yrs in 1980 with a 6 mth history of intermittent and then progressive right-sided visual failure; similar symptoms developed 2 mths later in the left eye. Examination in November 1980 showed no PL on the right with optic atrophy and reduced acuity of 1/36 with disc swelling on the left. There were no other neurological symptoms or signs.

The following year she had an episode of weakness in the right leg associated with impaired sensation which recovered. These symptoms recurred in 1983 and were associated with urinary urgency and frequency. Motor and sensory symptoms developed in the left leg during 1984; examination showed upper motor neuron signs in the right arm and both legs with impaired cutaneous sensation below T5. From that time her neurological symptoms have progressed slowly with the development of impaired mobility due to spasticity, weakness and ataxia; in addition to the features of optic nerve disease, examination has demonstrated abnormalities arising from the cervical cord and brainstem-cerebellar connections, including a left internuclear ophthalmoplegia.

Analysis of the CSF in 1980 showed a lymphocytic pleocytosis (23 cells per cubic millimetre) and increased IgG production. An MRI scan (July 1991) showed generalized cerebral atrophy, particularly affecting the corpus callosum, with ventricular dilatation and multiple areas of increased signal involving the periventricular white matter and centrum semi-ovale. Urodynamic assessment in 1986 showed evidence of an unstable bladder with early contractions and sphincter-detrusor dyssynergia.

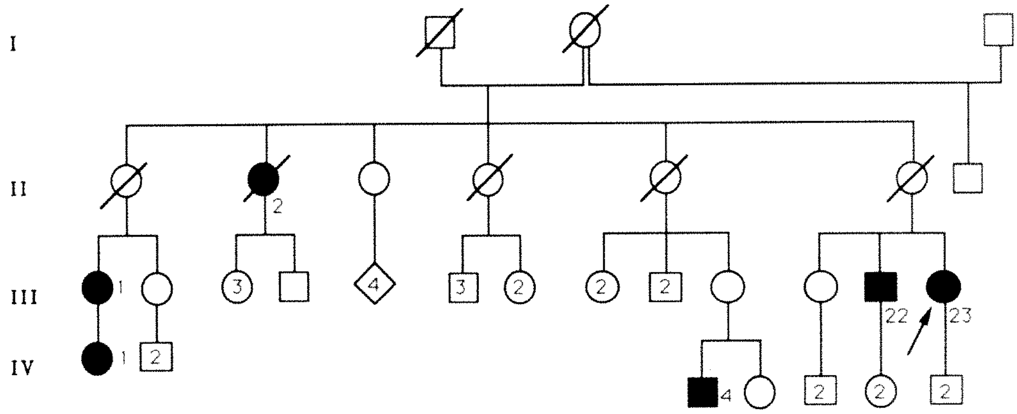

FIG. 3. Pedigree of family of *Case 3*. Circle = female; square = male; diamond = gender unspecified; oblique lines = deceased; filled in symbols = affected; open = unaffected; numbers in symbols = numbers of individuals; number below symbol = pedigree number (*see text*); arrow = proband (*Case 3*).

The patient's brother (III.22) lost vision in the left eye in mid-1974 (aged 30 yrs); this improved transiently but within 6 mths he had bilateral visual loss with acuity reduced to CF in both eyes, large central scotomas and optic atrophy. The CSF was normal. No symptoms or signs attributable to lesions outside the visual system had developed when he was last seen in 1980.

Her matrilineal second cousin (IV.4) developed gradual loss of vision in the right eye during 1981 (aged 19 yrs) leading to blindness in that eye within 1 mth. He improved after treatment with corticosteroids, but later developed progressive visual failure due to optic nerve disease but associated with right-sided choroiditis and retinal detachment which were thought to be coincidental. Visual symptoms developed and progressed rapidly in the left eye in 1982. Investigation showed normal CSF; antinuclear antibodies were weakly and transiently positive but there was no other evidence for systemic vasculitis. There had been no symptoms or signs indicating involvement outside the visual system when he was last seen in 1984.

Her matrilineal first cousin (III.1), a 60-yr-old female, has not been under neurological care but developed epilepsy in infancy and reported rapid loss of vision in both eyes at the age of 18 yrs, attributing this to an earlier overdose of quinine. In 1991 she was found to have severely reduced visual acuity with bilateral optic atrophy but there was no evidence for involvement outside the visual system. Her 27-yr-old daughter (IV.1) noticed visual symptoms in the right eye during 1991; examination showed reduced acuity of 6/24 on the affected side with a central scotoma and bilateral optic atrophy.

The patient's maternal aunt (II.2) was thought by the family to have had MS; she died at the age of 62 yrs but her medical records are not available.

Case 4. This 62-yr-old female was examined shortly after her 37-yr-old son had been diagnosed as having LHON. Her brother and maternal uncle had gone blind in their twenties. At the age of 34 yrs she abruptly developed diplopia, vertigo, nausea and unsteadiness. This lasted for several weeks and then recovered over about 6 mths. Seven years later she had an episode of patchy loss of vision and this recurred after a further 3 yrs. Two years later she developed severe visual loss, particularly in the right eye, which never recovered. She subsequently experienced episodes of vertigo and unsteadiness lasting about 3 mths, but had had no attacks for 2 yrs prior to assessment.

On examination visual acuity was CF on the right and 6/60 on the left, with bilateral optic atrophy. Pursuit eye movements were broken up and there was a right internuclear ophthalmoplegia. There was mild pyramidal weakness, more severe in the legs. The reflexes were brisker on the right than the left and both planter responses were extensor.

Case 5. This 56-yr-old woman was well until the age of 33 yrs when she noticed fogginess of vision, mainly in the central field of the left eye. She was unable to read. Poor vision persisted for several months but then improved gradually over about 1 yr. When she was 46 yrs old she had an episode of vertigo, vomiting and unsteadiness which lasted 6 wks; subsequently her balance was slightly impaired and she had urgency of micturition. In late 1990 she developed recurrent loss of vision, more on the right than the left, and was unable to read for several weeks. Vision started to improve after 3 mths but tended to fluctuate and was worse after a hot bath. The patient's mother developed severe visual loss at the age of 58 yrs but apparently made a good recovery. Her brother and a maternally related male cousin had been diagnosed as having LHON.

On examination visual acuity was 6/9 on the right and 6/6 on the left. Colour vision was grossly impaired. The optic discs were pale but fundal vessels were normal. Pursuit eye movements were jerky and there was nystagmus on right lateral gaze. The left hand was clumsy and the abdominal reflexes were absent; otherwise neurological examination was normal. Visual evoked potentials were delayed and reduced in amplitude. T_2-weighted MRI showed multiple extensive lesions in the cerebral white matter, most marked in periventricular regions, but also in the cerebellum and brainstem.

Case 6. This French woman had a strong family history of LHON; 10 male relatives in the maternal line (three great uncles, three uncles, two brothers and two nephews) had gone blind between the ages of 20 yrs and 30 yrs. She was well until the age of 32 yrs when she lost vision in the left eye; this did not recover and was followed after 12 yrs by similar problems in the right eye. On examination then, in 1986, visual acuity was 1/60 on the left with optic atrophy, and 2/60 on the right with a swollen disc. There were bilateral central scotomas. In 1990 she had an episode of numbness and weakness of the left arm which resolved within 3 wks after a course of intravenous methyl prednisolone. The CSF was normal. T_2-weighted MRI showed numerous high signal lesions in the cerebral white matter which were predominantly periventricular.

Case 7. This 25-yr-old Greek woman noticed mistiness of vision in the left eye in April 1986; progressive visual loss occurred over 4–5 wks and the right eye became similarly affected 3 wks after onset in the left. There had been no improvement up until September 1986. On examination then, visual acuity was confined to CF bilaterally. There was bilateral optic atrophy, more marked on the left, and telangiectatic vessels on the nasal half of the right disc. There were bilateral central scotomas. Neurological examination was otherwise unremarkable. The patient's brother, aged 31 yrs, had developed similar symptoms at the age of 17 yrs and had never recovered. On T_2-weighted MRI there were multiple cerebral white matter lesions in both periventricular and subcortical regions. Optic nerve imaging showed increased signal in both optic nerves in their posterior orbital and intracanalicular portions.

Case 8. This 59-yr-old woman was well until September 1973 when, at the age of 41 yrs, she noticed intermittent blurring of vision in the right eye. When examined 1 mth later visual acuities were 6/60 on the right, with optic atrophy, and 6/9 on the left. After a further 2 mths, vision in the right eye was persistently reduced and she then developed visual loss on the left over a period of 2 wks. On examination in January 1984 acuities were HM on the right and 3/60 on the left. Both discs were pale, particularly the right. There were large bilateral central scotomas. A CT scan of the orbits, air encephalography, right carotid angiography and CSF were normal. Pattern VEPs were absent. She was treated with steroids but 3 mths later visual acuity had deteriorated to CF on the left. Surgical exploration of the optic chiasm showed no compressive lesion.

Over the next 17 yrs she remained blind but developed no other neurological symptoms. Examination in 1991 showed bilateral optic atrophy and detection of HM only in the periphery of both visual fields; the rest of the neurological examination was normal. T_2-weighted MRI showed multiple small periventricular and subcortical lesions. The appearances were non-specific, being compatible with either MS or vascular disease. The patient's son developed bilateral sequential visual loss over a period of 4 wks in early 1990 and has not recovered, with visual acuities of 2/60 bilaterally in 1991. There was no other relevant family history.

RESULTS

The 11778 bp mutation was present in at least 95% of mtDNA extracted from blood in all eight patients and all of their affected relatives available for study (the brother of Case 1, the son and brother of Case 4, the son of Case 8, and three relatives of Case 3, III.1, IV.1 and IV.4 in Fig. 3). Cases 4 and 7 were members of families reported earlier (Holt *et al.*, 1989).

HLA DR/DQ genotyping was performed in six of the patients and the affected brother of Case 1, with results as follows: Case 1 and her brother, DR2 (DRw15), DR7, DQw6, DQw2; Case 2, DR2 (DRw15), DR7/9, DQw6, DQw9; Case 3, DRw6 (DRw13), DR7, DQw6, DQw2; Case 5, DR2 (DRw15), DQw6 (homozygous); Case 7, DR3 (DRw17), DRw6 (DRw14), DQw2, DQw5; Case 8, DR4, DQw8 (homozygous).

DISCUSSION

The clinical features of Cases 1–6 in this series are those of an illness indistinguishable from MS, as defined by the Poser criteria (Poser *et al.*, 1983), occurring in the context of a family history of LHON associated with the 11778 bp mtDNA mutation. The presence of epilepsy as a presenting feature in Case 2 may have been coincidental. The clinical diagnosis of MS was supported by the finding of widespread white matter lesions on MRI in all the patients examined, and abnormalities of CSF and evoked potentials were observed in most of those investigated. In Cases 7 and 8 the clinical signs were confined to the optic nerves, but both had multiple white matter lesions on MRI. In view of the age of Case 8 these could have been due to vascular disease, but she was only 59 yrs old and there were no vascular risk factors. One particularly striking feature of this series is that all the patients were female. We have not observed other neurological abnormalities in over 50 males with LHON, and brain MRI performed in 13 of them was normal (Kermode *et al.*, 1989). Furthermore, we have performed MRI in one other female with LHON and the 11778 bp mutation, and she had a single cerebral white matter lesion; the two remaining affected women in our total LHON population with this genetic defect have no other neurological features but have not been scanned.

The diagnosis made at presentation in these patients was usually bilateral simultaneous or sequential optic neuritis. This interpretation was often supported by the subsequent development of other symptoms and signs suggesting MS. This is not surprising given that LHON is rarely considered as a cause of blindness in women and that the family history of visual failure emerged later in many cases. Sequential optic neuritis is common in MS and substantial recovery usually occurs. With one exception in this series (Case 5), visual loss was severe and persistent, an observation suggesting that the optic nerve disease was due to LHON rather than a feature of MS. Whether these patients have LHON and MS, or a different disorder, is uncertain. It is highly unlikely that these observations represent a coincidental occurrence given the population frequencies of MS (one in 800) and LHON (probably about one in 50 000 overall but one-fifth of this figure for females).

An association between LHON and a variety of other neurological disorders was suggested in the early literature; many of these reports can be dismissed as the diagnosis of LHON was clearly, in retrospect, incorrect (Wilson, 1963). Patients fulfilling current diagnostic criteria for LHON and clinically definite MS include one reported by Lees *et al.* (1964) and another by Ferguson and Critchley (1928); these were both female. Two male patients with LHON and the 11778 bp mutation reported by Newman *et al.* (1991) had a family history of MS, but the sex of these relatives was not stated. The cases described by Wilson (1963) had clinical and pathological features making MS highly unlikely, including prominent dementia, extrapyramidal and anterior horn cell disease. Thus this association appears to be rare. However, the diagnosis of each disorder was difficult to confirm without modern methods of investigation such as MRI, CSF immunoglobulin electrophoresis, and, in the case of LHON, mtDNA analysis.

These observations prompt speculation about the role of immunological factors in the pathogenesis of LHON, and also that of mitochondrial genes in MS. Susceptibility to MS is multifactorial, involving an interplay between genes encoded at different loci and environmental triggers (Compston, 1991). The details have yet to be resolved, but there is evidence for involvement of one or more genes encoded in the class II region of the histocompatibility complex locus on chromosome 6, the closest association being with the tightly linked alleles DRB1.0501 and DQB1.0602, subtypes of DR2/DQw6. It is of interest that four of six patients investigated here typed as DR2 and/or DQw6; these alleles occur in about 30% of the caucasian population (Olerup and Hillert, 1991). Another susceptibility gene has been identified in the T cell receptor locus on chromosome 7, which is provisionally located centromeric to VB11 (Charmley *et al.*, 1991). These genes do not account entirely for the degree of heritability implicated in several population based studies of MS, and it is presumed that other as yet unidentified genes contribute to susceptibility; these could include one or more mitochondrial genes. In favour of this hypothesis is the observation that females with MS are more likely to have affected children than males, even taking the female preponderance of MS into account. However, there is no simple interpretation for the pedigrees observed in multiplex families with MS and a mitochondrial genetic hypothesis would not explain the excess of affected daughters irrespective of parental sex in affected parent-offspring pairs (Sadovnick *et al.*, 1991).

The genes which have so far been implicated in determining susceptibility to MS all encode products which play a key role in restriction of the immune response. Their polymorphic protein products are involved in transport, presentation and recognition of antigenic peptide fragments; although it is possible that genetic factors of a non-immunological nature are relevant to the pathogenesis of MS, none has so far been identified. Thus if mitochondrial genes are involved it is tempting to speculate that their effects are immunologically mediated. There is evidence that these genes play a part in the immune response in rodents, in the form of a maternally transmitted minor murine histocompatibility antigen which is encoded by mtDNA (maternally transmitted factor: MTF; Fischer Lindahl and Bürki, 1982; Loveland *et al.*, 1990). The ND1 gene appears to determine MTF antigenicity.

In LHON, the 11778 bp mutation is clearly associated with the disease in many families, but its presence does not appear to be the sole determinant of the phenotype; some family members with a high proportion of mutant mtDNA remain unaffected. In particular, it does not explain the relative paucity of affected females. An X-linked visual loss susceptibility gene has recently been suggested on the basis of linkage studies, affected females needing to be homozygous for the susceptibility allele which appears to be common in the general population (Vilkki *et al.*, 1991). These observations have not been confirmed in families in the UK (Sweeney *et al.*, 1991). It is also not clear how a fairly conservative amino acid change in a respiratory chain subunit causes subacute blindness in young adult life. There are several clinical observations in LHON which are surprising for a genetic disease. The onset of blindness is subacute and the optic disc appearances at the time of presentation are reminiscent of an inflammatory microangiopathy, although it is notable that the vessels do not usually leak fluoroscein.

Many patients have increased immunoglobulins in their CSF (Pallini *et al.*, 1988). These observations, and the patients presented here, suggest that immunological factors could be relevant in the pathogenesis of LHON.

It is not clear how mitochondrially peptides encoded by mtDNA are able to play a role in the immune response. One question, how they reach the cell surface, is unresolved, although several hypothetical mechanisms have been proposed by Loveland *et al.* (1990). Furthermore, epitopes recognized by helper and cytotoxic cells generally contain a charged residue or glycine followed by two hydrophobic residues (Rothbard and Taylor, 1988); the amino acid sequence in ND1-MTF does not carry this pattern in any of its allelic forms. In fact, the N-terminal sequence, which confers cytotoxicity, is strongly hydrophobic and contains no charged residues. The conservation of this sequence does suggest an important role, possibly in anchoring ND1 in the lipid membrane. The amino acid changes observed in the polymorphisms of ND1-MTF may alter the orientation of the extramembranous residues to induce cytotoxic activation. Although it seems unlikely that the part of ND4 affected in LHON by the 11778 bp mutation is homologous to MTF, it is possible that the resulting amino acid change similarly elicits an abnormal, tissue specific, immune response which could effect the development of blindness in LHON. The mutation converts a conserved arginine at position 340 to a histidine; both these amino acids are basic and hydrophilic, are likely to be part of an extramembranous sequence, and as such may form an antigenic determinant. It is of interest that the two other, less common, mtDNA mutations associated exclusively with LHON each give rise to an amino acid substitution in a hydrophilic loop of the ND1 protein and could thus generate auto-antigens (Howell *et al.*, 1991*b*).

Pathogenic mtDNA mutations are sometimes associated with circulating antibodies directed against mitochondrial proteins (Schapira *et al.*, 1990). A specific antibody to a 41 kDa mitochondrial matrix protein was observed in a patient with the MELAS (mitochondrial encephalopathy, lactic acidosis and strokelike episodes) phenotype of mitochondrial myopathy. This patient had the MELAS mtDNA mutation in the $\mathrm{tRNA}^{\mathrm{Leu}}$ gene (Goto *et al.*, 1990; Hammans *et al.*, 1991). Suborganellar fractionation and immunoblotting studies suggested that the 41 kDa antigen was a matrix protein and not part of the respiratory chain. As such the 41 kDa protein would be encoded by a nuclear gene. The generation of antibodies to mitochondrial proteins encoded by nuclear genes could arise either from incorporation of the protein into the plasma membrane following autophagic recycling of mitochondria, or mistrafficking of mitochondrially targeted proteins to the plasma membrane. The relevance of the auto-antibody to this patient's disease is difficult to establish. The same applies to the antibodies to mitochondrial proteins which occur in primary biliary cirrhosis (PBC). The mitochondrial M2 subclass of antibodies in PBC is directed against a heterogeneous group of antigens, the most important of which are subunits of pyruvate dehydrogenase (Yeaman *et al.*, 1988). It is of interest that steroid responsiveness has been described in MELAS patients, suggesting that immunity may play some part in the development and expression of this disease which is associated with a point mutation in the mtDNA $\mathrm{tRNA}^{\mathrm{Leu}}$ gene in most cases.

In conclusion, clinical and experimental observations lead to the hypothesis that visual failure in LHON may be produced by abnormal immune responses directed against the optic nerves, arising directly or indirectly from mutations of mtDNA. In some individuals with the 11778 bp mutation, particularly females, this process may involve other myelinated axons in the central nervous system, producing a disorder indistinguishable from MS. It is thus possible that mitochondrial genes contribute to susceptibility to MS. From the clinical point of view, our observations indicate that mtDNA anlysis should be performed in women, as well as men, who present with simultaneous or sequential bilateral optic neuropathy, even if there is no family history of subacute visual failure or other neurological features develop later. Apart from the diagnostic benefit of demonstrating any of the mtDNA mutations associated with LHON, there are important implications for genetic counselling.

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TREATMENT OF EXPERIMENTAL NADH UBIQUINONE REDUCTASE DEFICIENCY WITH MENADIONE

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SUMMARY

Chronic administration of diphenylene iodonium (DPI) to rats has been shown to model the characteristics of mitochondrial myopathy. Using this model the efficacy of menadione therapy has been assessed. Menadione treatment of rats injected with DPI was associated with improved weight gain and increased survival rate. This was accompanied by an improvement in muscle function as judged by analysis of isometric twitch tension of the gastrocnemius muscle (1 Hz for 20 min). The decline in phosphocreatine (PCr) levels in the gastrocnemius muscle during stimulation and delayed recovery in PCr after stimulation were similar in the menadione treated and untreated models. Menadione treatment of the DPI model resulted in a resting intramuscular pH significantly lower than control or untreated DPI rats, but a similar decline in intramuscular pH to the DPI rats during stimulation. The changes in metabolite levels were broadly similar in both the menadione treated and untreated DPI models following stimulation, although the changes, except for increased lactate concentration, were generally less marked in the menadione-treated DPI model.

INTRODUCTION

Disorders of the mitochondrial respiratory chain have been described in a variety of clinical conditions grouped together by the term 'mitochondrial myopathies' (for reviews, *see* DiMauro *et al.*, 1985; Morgan-Hughes, 1986). Defects of each of the respiratory complexes have been reported, but impairment of complex I (NADH ubiquinone reductase) activity has been a common observation (Koga *et al.*, 1988; Cooper *et al.*, 1990). To date no consistently effective treatments have been identified either in man or animal models, but the potential to bypass complex I makes menadione a candidate for use as a treatment for patients with a defect at the level of NADH ubiquinone reductase. Eleff *et al.* (1984) used ^{31}P nuclear magnetic resonance (NMR) to assess the treatment, involving menadione and ascorbate, of a patient with a defect of ubiquinol-cytochrome *c* reductase (complex III). After treatment they reported improvements in the clinical condition of the patient, which was supported by increased muscle phosphocreatine concentration both at rest and during recovery after exercise. Wijburg *et al.* (1990) used fibroblasts inhibited maximally with rotenone to assess menadione

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therapy. Menadione improved the lactate to pyruvate ratios and partially restored glucose oxidation and adenosine triphosphate (ATP) levels.

A rat model with similar biochemical characteristics to those seen in the human condition of complex I deficiency has been developed by chronic injection of the complex I inhibitor diphenylene iodonium (DPI) (Cooper *et al.*, 1988*a*).

Menadione (vitamin K_3) has been shown to increase oxygen utilization by rat liver mitochondria (De Groot *et al.*, 1985) inhibited by rotenone (a known complex I inhibitor). Cyanide, dicoumarol and antimycin A have all been shown to inhibit this phenomenon to varying extents, implying that both the mitochondrial respiratory chain and DT diaphorase are contributing to menadione-stimulated oxygen utilization. One potential problem associated with menadione therapy is its ability to generate oxgen radicals (Smith *et al.*, 1985), and subsequent damage to the cell. However, De Groot *et al.* (1985) have calculated that in liver mitochondria >90% of the menadione-dependent oxygen utilization results in the production of H_2O and very little production of the superoxide free radical O_2^-.

Although DPI inhibits complex I at a different site to rotenone (Ragan and Bloxham, 1977) a menadione-dependent increase in oxygen utilization has been observed in liver mitochondria inhibited with DPI, similar to that reported for rotenone inhibition (Holland *et al.*, 1973).

This model has been used to investigate the potential therapeutic effects of menadione on complex I inhibition and assess its effects using ^{31}P NMR in conjunction with physiological and biochemical analyses of the gastrocnemius muscle.

METHODS

Male Wistar rats (20 d old) were injected daily (subcutaneously) with DPI (1.5 mg/kg body weight) for 5–6 wks to produce the chronic DPI model (Cooper *et al.*, 1988*a*). Half of the DPI-treated rats were injected daily with 10 mg menadione per kilogram body weight (calculated to obtain a maximum menadione concentration of 50 μM assuming a body water content of 700 ml/kg and 100% absorption) and half with vehicle alone. Control rats were injected with menadione alone (10 mg/kg)

Isometric twitch tensions were determined as described by Byrne *et al.* (1985) using the stimulation protocol of 1 Hz for 20 min followed by 5 Hz for 10 min. The stimulated and contralateral muscles were freeze clamped at the end of stimulation, extracted and assayed for metabolites as described by Cooper *et al.* (1988*a*). Liver and gastrocnemius muscle mitochondria were freshly isolated and polarographic and enzymatic analyses performed as described by Cooper *et al.* (1988*a*).

^{31}P NMR studies were conducted on the gastrocnemius region of the rat hind limb as described by Cooper *et al.* (1988*b*).

RESULTS

After 5 wks the body weights of the menadione-treated DPI model (255 ± 10 g, n = 12) were significantly ($P \leq 0.001$) greater than those of the untreated model, (213 ± 19 g, n = 12), but still significantly ($P \leq 0.001$) below control values (300 ± 25 g, n = 12). There were no deaths due to DPI treatment in those rats treated with menadione in comparison to a 11% (2 out of 18) death rate in the untreated DPI model.

At 1 Hz stimulation of the gastrocnemius muscle, the decline in isometric twitch tension observed in the DPI model (Cooper *et al.*, 1988*b*) was absent in the menadione-treated model. Upon 5 Hz stimulation for a further 10 min, the 'staircase' in isometric twitch tension observed in controls but absent from the DPI-injected rats, was restored in the DPI rats treated with menadione. The subsequent decline in isometric twitch tension was graduated in the order DPI > DPI plus menadione > control plus menadione (Fig. 1).

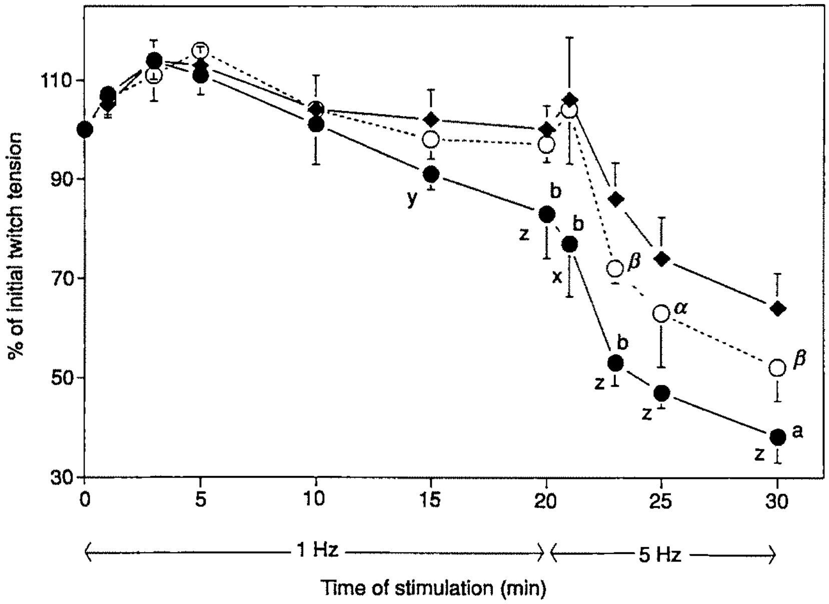

FIG. 1. Analysis of the isometric twitch tension of the gastrocnemius muscle in rats injected with menadione (◆), DPI (●), or DPI and menadione (○) Values are mean ± SD (n = 5) Initial isometric twitch tensions were 1.89 ± 0.13, 1.80 ± 0 12, 1 68 ± 0 21 gm force/kg body weight, respectively. Student *t* test;- $P < 0.05$, $P < 0.01$, $P < 0.001$ denoted as α, β, τ for DPI+menadione vs control+menadione, as a, b, c for DPI vs DPI+menadione, as x, y, z for DPI vs control+menadione.

^{31}P NMR analysis of the gastrocnemius muscle revealed a decline in the PCr/(PCr+P_i) (P_i = inorganic phosphate) ratio of both the menadione treated and untreated DPI model during stimulation at 1 Hz, and a rapid decline during 5 Hz stimulation to values significantly below the control values. The recovery of the PCr/(PCr+P_i) ratio at the end of the stimulation protocol was delayed to a similar extent in both menadione-treated and untreated models (Fig. 2). In the menadione-treated model the intramuscular pH at rest was significantly lower than that of both the untreated model and control rats. During 1 Hz stimulation the decrease in intramuscular pH observed in the untreated model was greater in the menadione-treated DPI model, although this was not statistically significant (Fig. 3).

The lower resting intramuscular pH in the menadione-treated model was consistent with the increased lactate concentration in the unstimulated contralateral muscle (Fig. 4F). In contrast, however, the unstimulated contralateral gastrocnemius muscle of the

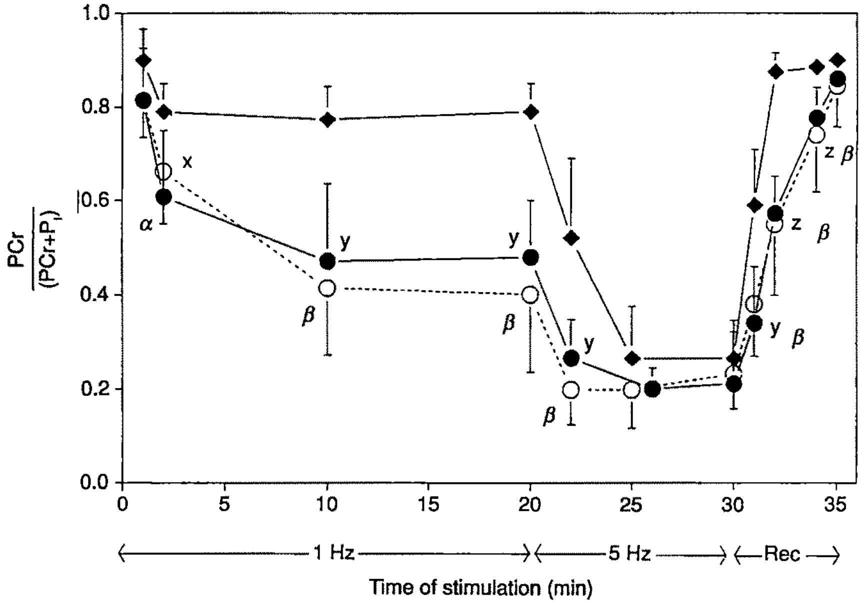

FIG 2 ^{31}P NMR analysis of the rat hind limb during stimulation and recovery in rats injected with menadione, n = 5 (◆), DPI, n = 4 (●), or DPI and menadione, n = 5 (○). Phosphocreatine and P_i concentrations were measured as described in the Methods and expressed as a ratio of PCr/(PCr+P_i) Student t test as Fig. 1.

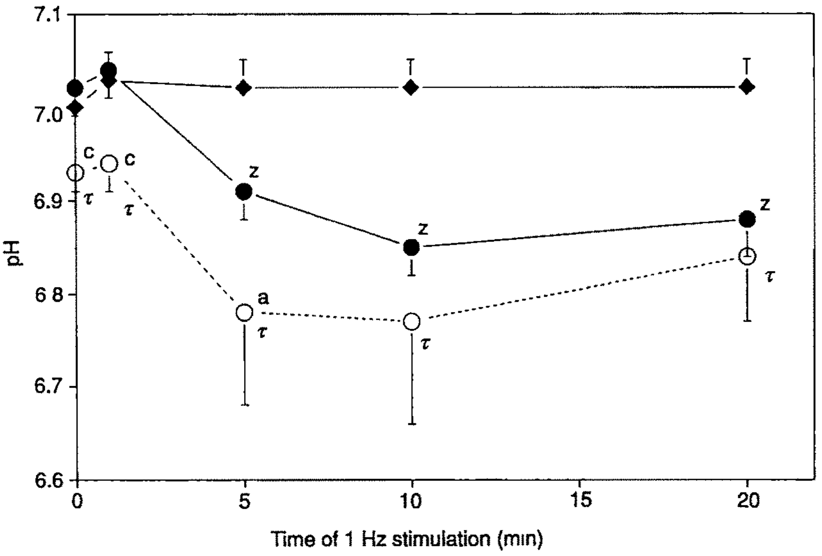

FIG. 3. Intra-muscular pH of the rat hind limb during 1 Hz stimulation in rats injected with menadione, n = 5 (◆), DPI, n = 4 (●), or DPI and menadione, n = 5 (○) Intramuscular pH was calculated from ^{31}P NMR spectra as indicated in the Methods section Student t test as Fig. 1.

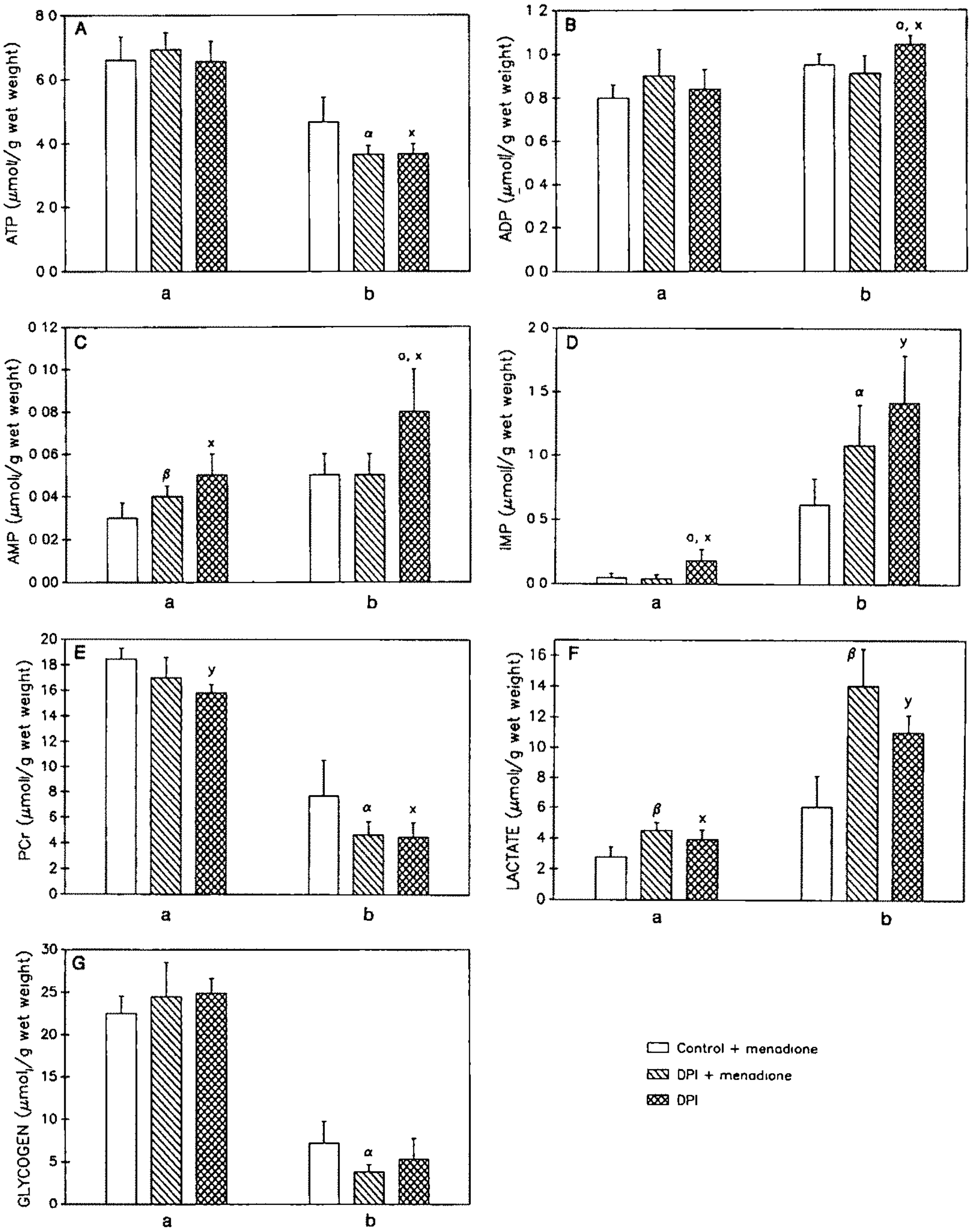

FIG. 4 Metabolite levels in the rat gastrocnemius muscle freeze-clamped at the end of stimulation (1 Hz for 20 min plus 5 Hz for 10 min). a, contralateral unstimulated and b, stimulated muscle Values are mean ± SD, control (n = 5), DPI+menadione (n = 4), DPI (n = 4). Student *t* test as Fig 1

menadione-treated model showed lower levels of inosine monophosphate (IMP) (Fig. 4D) and higher levels of PCr (Fig. 4E).

At the end of the stimulation protocol the metabolite content of the gastrocnemius muscles from the menadione-treated and untreated DPI models were similar, differing from the control rats in the following respects; decreased adenosine triphosphate (ATP) and PCr levels, and increased lactate levels (Fig. 4A,E,F). However, in the menadione-treated model both adenosine monophosphate (AMP) (Fig. 4C) and adenosine diphosphate (ADP) (Fig. 4B) levels were significantly lower and IMP was lower (Fig. 4D), although not significantly, in comparison to the untreated model.

The free ADP concentrations were calculated from the creatine kinase equilibrium as described by Arnold *et al.* (1985), using the intramuscular pH values at rest (Fig. 3) and the metabolite levels of the unstimulated muscles (Fig. 4). This gave values of 7.7 ± 0.8 μM, 13.4 ± 1.4 μM and 21.4 ± 2.2 μM for controls+menadione, DPI+menadione and DPI alone, respectively.

Isolated mitochondria from the gastrocnemius muscle and from the liver revealed no restoration by menadione of the inhibition of NADH-linked substrate oxidation caused by DPI (Table 1). Enzymatic analysis showed a small increase in the NADH ferricyanide reductase activity. The activities of glycogen phosphorylase and lactate dehydrogenase were decreased in the chronic DPI model (Cooper *et al.*, 1988*a*; Table 2), but after menadione treatment these activities were restored to control values (Table 2).

The biochemical, physiological and NMR results obtained for the control rats injected with menadione did not differ significantly from control rats published previously (Cooper *et al.*, 1988*a,b*).

DISCUSSION

The therapy of patients with mitochondrial abnormalities has received little attention in the literature, many reports being little more than anecdotal. Using the chronic DPI rat model (Cooper *et al.*, 1988*a*), this study has attempted to assess the efficacy of menadione treatment of mitochondrial complex I dysfunction. It was chosen to study muscle because not only can it be investigated using physiological, biochemical and NMR techniques, but these parameters have already been shown to be affected by decreased mitochondrial activity (Cooper *et al.*, 1988*b*). In addition to this it has been shown that a 60% inhibition of complex I activity in the gastrocnemius muscle (1.5 mg DPI/kg body weight) results in muscle fatigue at 1 Hz stimulation. However, when complex I activity was only inhibited 40% (1 mg DPI/kg body weight), fatigue was not apparent at 4 Hz stimulation (Cooper *et al.*, 1988*b*). Consequently it can be postulated that menadione treatment of the 1.5 mg DPI/kg body weight model need only improve mitochondrial function partially for this to be reflected as an improved muscle physiological response.

Isometric twitch tension analysis of the gastrocnemius muscle demonstrated an improvement in muscle function with menadione therapy under 1 Hz stimulation. However, this was accompanied by a decreased intramuscular pH and associated increase in intramuscular lactate concentration. The fall in PCr during muscle stimulation and delayed recovery after stimulation, consistent with a defect of the mitochondrial respiratory chain, did not improve with menadione therapy, suggesting that the

TABLE 1 OXYGEN UTILIZATION OF GASTROCNEMIUS MUSCLE (A) AND LIVER (B) MITOCHONDRIA ISOLATED AFTER FIVE WEEKS OF INJECTIONS

Substrate	*Control+ menadione*	*DPI+ menadione*	*DPI*
(A)	*(n = 7)*	*(n = 7)*	*(n = 5)*
Glutamate	180 ± 27	62 ± 28^{r}	47 ± 19^{z}
Succinate	183 ± 21	174 ± 28	164 ± 16
Ascorbate+TMPD	422 ± 130	377 ± 96	395 ± 38
(B)	*(n = 3)*	*(n = 3)*	*(n = 3)*
Glutamate	57 ± 8	26 ± 4^{r}	24 ± 6^{z}
Succinate	89 ± 15	104 ± 4	92 ± 27
Ascorbate+TMPD	97 ± 21	120 ± 12	115 ± 25

Values are mean ± SD, nano g atoms O/min/mg mitochondrial protein. Statistical analysis as for Fig. 1

TABLE 2. MITOCHONDRIAL RESPIRATORY CHAIN ACTIVITIES OF MITOCHONDRIA ISOLATED FROM THE GASTROCNEMIUS MUSCLE* AND GLYCOLYTIC ENZYME ACTIVITIES OF THE WHOLE GASTROCNEMIUS MUSCLE**

	Control+ menadione (n = 4)	*DPI+ menadione (n = 4)*	*DPI (n = 4)*
NADH ferricyanide reductase*	2355 ± 369	963 ± 240^{r}	$624 \pm 77^{a,z}$
NADH cytochrome *c* reductase*	215 ± 30	78 ± 13^{r}	ND
Hexokinase**	1.81 ± 0.69	1.97 ± 0.70	1.70 ± 0.19
Phosphofructokinase**	38.6 ± 4.1	43.6 ± 3.4	41.2 ± 2.9
Glycogen phosphorylase**	52.1 ± 5.7	51.9 ± 6.3	$43.4 \pm 2.2^{a,x}$
Lactate dehydrogenase**	530 ± 68	608 ± 47	$430 \pm 39^{a,x}$

*nmol/min/mg mitochondrial protein, **μmol/min/g wet weight of muscle ND = not determined. Values are mean ± SD. Statistical analysis as for Fig. 1

improvement in isometric twitch tension under this stimulation could not be attributed to a more efficient ATP generation from oxidative phosphorylation.

In the menadione-treated model, although the decline in isometric twitch tension (1+5 Hz) was not as marked as in the untreated DPI model, the resultant changes in metabolite levels were similar to the untreated model, suggesting there was little improvement in aerobic energy production. Lactate concentrations were higher in the menadione-treated DPI-treated rats, suggesting anaerobic glycolysis may have been stimulated in these rats, possibly facilitating increased ATP production and improved muscle performance. There have been reports in hepatocytes that menadione may stimulate glycolysis (Smith *et al.*, 1987) or cause depletion of ATP by a combination of uncoupling oxidative phosphorylation and by decreasing the flux through glycolysis (Redegeld *et al.*, 1989). Levels of ATP were not affected in the unstimulated contralateral gastrocnemius muscle by menadione treatment (Fig 4A), suggesting menadione may stimulate or depress glycolytic ATP synthesis in a tissue-specific manner. Since in the chronic DPI-treated rat a number of tissues exhibited an inhibition of mitochondrial function (including skeletal muscle, heart, liver), the general improvement in health and survival of the DPI animals treated with menadione may involve improvements in aerobic ATP generation or increased glycolysis in tissues other than skeletal muscle.

The hypothesis behind the use of menadione as a therapy for defects of mitochondrial complex I is that menadione has been shown to stimulate oxygen utilization in mitochondria using NAD-linked substrates in the presence of the complex I inhibitors rotenone (De Groot *et al.*, 1985) or DPI (Holland *et al.*, 1973). Treatment with menadione should result in increased aerobic NADH oxidation, thereby decreasing the production of lactate and, because both sites II and III of oxidative phosphorylation should be involved in the bypass of complex I inhibition, ATP synthesis should be restored at least in part, resulting in improved cell function. The failure of menadione to achieve either improved oxidative phosphorylation as suggested by the abnormal ^{31}P NMR results (Fig. 2), or to decrease lactate levels (Fig. 4F), does not support this hypothesis. This may reflect an inability of the menadione to bypass a complex I inhibition *in vivo* in skeletal muscle or at least so minimally that it is not detectable using these techniques. The menadione bypass pathway has been demonstrated in liver (De Groot *et al.*, 1985) and heart (Dedukhova *et al.*, 1986), but it has not been investigated in skeletal muscle.

It was not possible to determine the cellular menadione concentration in this study; however, there is indirect evidence that menadione reaches the cells at effective concentrations in rats treated in the same manner as in this study (White and Clark, 1988).

The data from this study are in contrast to the findings of Wijburg *et al.* which may reflect the different models and conditions used. Wijburg *et al.* (1990) used a fibroblast cell line effectively at rest and inhibited maximally by rotenone. This had the consequences of markedly increasing the lactate : pyruvate ratio and depleting the ATP concentrations. From this background small increases in aerobic energy metabolism would be more easily observed. Complex I was inhibited 60% in the muscle of the DPI model and there were no dramatic biochemical changes at rest; however, the menadione-induced improvement in the DPI animals' general health could be due to an improvement in aerobic energy metabolism similar to that observed in the fibroblast model. The absence of any indicators of improved aerobic energy metabolism during muscle stimulation in the menadione-treated DPI model suggests the menadione-induced improvements in aerobic energy metabolism are either absent or insignificant under conditions of high energy turnover. The improvement in muscle function in the menadione-treated DPI model could be due to undetectable improvements in oxidative metabolism. Improved (i.e. lowered) AMP (Fig 4C), IMP (Fig. 4D) and free ADP levels suggests the changes in IMP and free ADP concentrations, which have been previously correlated with muscle performance (Arnold *et al.*, 1985; Westra *et al.*, 1986), may contribute to the improved muscle performance observed with menadione therapy. The increased lactic acidosis (Fig. 4F) and decreased intramuscular pH (Fig. 3) with no improvement in phosphocreatine concentrations (Fig. 2, Fig. 4E) suggests that the improvement in energy status and hence in muscle performance is either via glycolysis rather than oxidative phosphorylation, or indirectly via the improved situation in another organ, e.g. liver. The mechanisms for this could be an activation of key glycolytic enzymes in the muscle (Table 2), or by substrate supply to the muscle as a result of an effect of menadione elsewhere in the body.

In conclusion, although the DPI animal model has notable differences to the human condition with a complex I deficiency, this study suggests that menadione may be a beneficial therapy, allowing some patient improvement but at the expense of generating higher lactate concentrations with the associated lactic acidosis. Further studies are required to investigate the effects of menadione on other organs (e.g. central nervous system, liver) in order to determine whether the mechanisms of menadione therapy is acting directly on muscle or indirectly on other organs.

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HIPPOCAMPAL VOLUMETRIC AND MORPHOMETRIC STUDIES IN FRONTAL AND TEMPORAL LOBE EPILEPSY

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SUMMARY

The most common temporal lobe pathology is Ammons Horn sclerosis (AHS), and several different imaging techniques have been utilized to detect this with varying success. We describe the clinical application of magnetic resonance imaging (MRI) using a three-dimensional volume technique which allows total hippocampal volume to be measured and symmetry evaluated. Hippocampal surface area was calculated in sequential 1.5 mm thick contiguous images, using a GE IC workstation. Total volumes and surface areas were calculated. The cross-sectional surface area at 1.5 mm intervals was displayed graphically, permitting morphometric analysis of the hippocampus throughout its length. Focal atrophy within any part of the hippocampal formation (HF) and its extent could thus be assessed. Patients with well-lateralized temporal lobe epilepsy (TLE) (n = 20) and well-defined frontal lobe epilepsy (FLE) (n = 20) were studied, and volumes compared with normal values derived from 10 neurologically normal controls. Asymmetric hippocampal volume loss was demonstrated in all 20 patients with clinically typed TLE, but not in normal controls or patients with FLE. Volume loss distribution was anterior in 12 patients, posterior in one patient and widespread in seven patients. Secondarily generalized seizures were strongly associated with widespread loss.

This method of surface area and volumetric analysis of the hippocampus in TLE can demonstrate asymmetry and focal involvement, and help distinguish between hippocampal and frontal pathologies.

INTRODUCTION

Volumetric magnetic resonance imaging (MRI) studies of the hippocampal formation (HF) in temporal lobe epilepsy (TLE) have been applied to subjects with intractable complex partial seizures, relating asymmetrical volume loss to lateralized pathology (Jack *et al.*, 1990; Ashtari *et al.*, 1991). Other studies have normalized volume measurements, relative to total intracranial volume, and expressed a normal range of right-left HF volume differences (Jack *et al.*, 1989, 1990). These studies were of surgical candidates for temporal lobectomy, and subjects were excluded where alternative pathology was demonstrated in the surgical specimen or by imaging studies (Jack *et al.*, 1990). More recently, a study using fast low-angle shot imaging and computerized mensuration has demonstrated significantly reduced hippocampal volumes ipsilateral to the seizure focus in TLE (Ashtari *et al.*, 1991).

Previous studies have shown that hippocampal volume loss correlates well with clinical

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and electroencephalogram (EEG) seizure lateralization (Ashtari *et al.*, 1991; Bronen *et al.*, 1991; Cascino *et al.*, 1991). Pathological studies of hippocampal cell loss in TLE have established a relationship of volumetric changes and altered signal characteristics to cell loss and gliosis (Kuzniecky *et al.*, 1987; Lencz *et al.*, 1990; Bronen *et al.*, 1991; Cascino *et al.*, 1991). Pathological series of hippocampal changes in frontal lobe epilepsy (FLE) are not available. More recently, cell loss has been demonstrated in a group of patients with extra-hippocampal temporal lobe lesions. Most of these patients had basal temporal lesions, and histologically heterotopias and hamartomas were well represented (Lévesque *et al.*, 1991).

It has been suggested that frequent seizures generated at extra-temporal sites may also cause hippocampal cell loss. If so, HF volume asymmetry due to secondary hippocampal changes, resulting from extra-temporal lesions, would limit the value of such MRI volumetric studies in distinguishing between temporal and extra-temporal foci.

We have specifically addressed these problems. The aim of this study was first to develop a unique method of HF volume measurement which allows comparison between right and left and the detection of focal atrophy within the HF. The method is simple, accurate and reliable. It was then applied to patients with TLE, FLE and normal controls to identify how useful volume changes are in detecting atrophy in TLE, and also whether FLE causes lateralized HF volume changes.

PATIENTS AND METHODS

Patients and normals

Ten neurologically normal volunteers were examined as the control group (six male, four female, age range 21–36 yrs, average 30 yrs) (Table 1). Informed consent was obtained from all subjects. Twenty patients with FLE and 20 with TLE were selected on the basis of clinical features, EEG and video telemetry (Tables 2, 3). The average age of the TLE patients was 32 yrs (range 17–56 yrs), nine female, 11 male. Patients with TLE were categorized into those with frequent secondarily generalized seizures (patients who had more than 10 secondarily generalized seizures), and those without frequent secondary generalization. The FLE patients had a mean age of 30 yrs (range 18–61 yrs), nine male, 11 female. All patients with TLE had a normal cerebral computerized tomography scan as a condition of entry into the study. All patients had clear localization and lateralization on clinical and EEG information, and were prospectively acquired. One patient with FLE was excluded due to a poor quality scan (movement artefact), but no other subjects were excluded. All patients with TLE had clinical and EEG features strongly suggestive of lateralized mesial temporal onset, and none were discordant for clinical, EEG or psychological data. Ten of the FLE patients had a recognized extra-temporal lesion detected on MRI, and none showed significant intellectual decline, which might suggest more widespread pathology. Thus the study group comprised well-defined, uncomplicated unilateral cases. All subjects were right handed.

Magnetic resonance imaging was performed on a GE 1.5 Tesla Signa unit (GE Medical Systems, Milwaukee, USA). Volumetric imaging was performed in the coronal plane, using a spoiled gradient echo technique (SPGR). Contiguous 1.5 mm thick slices were obtained of the entire head, with a 35/5/1 (TR/TE/NEX) pulse sequence, flip angle 35 degrees, matrix size 256×128. This generated approximately 120 satisfactory images. Imaging time for this sequence was 9.5 min. In addition, routine sagittal T_1 (500/10/2 TR/TE/NEX), axial T_2 (2800/90/1 TR/TE/NEX) and proton density weighted (2800/30/1 TR/TE/NEX) series were obtained. All these studies had a 5 mm slice thickness and a 2.5 mm interslice gap. Spatial presaturation was employed. Field of view was 24 cm, matrix size 256×192 for routine studies. Total scanning time was 40 min. A pilot study was carried out, using identical routine parameters, but imaging only the temporal lobes with a SPGR sequence, 35/5/2 (TR/TE/NEX), however, the 60 images were not of sufficient quality, due to head movement and foldback artefact.

TABLE 1 NORMAL SUBJECTS

Normal subjects sex/age	*HF volumes (mm³) (right/left)*	*HF ratio (smaller/greater)*
1 M/30	3712/3700	0 99
2 F/24	3238/3364	0 96
3 F/27	3010/3095	0.97
4. M/28	2658/2683	0.99
5. M/30	2595/2664	0.97
6 M/31	3607/3700	0 97
7. F/36	3486/3632	0.96
8. F/24	3301/3222	0.98
9 M/21	2841/2856	0 99
10. M/36	3406/3375	0 99

Image processing

The images were transferred to a GE IC workstation (GE Medical Systems, Milwaukee, USA), where measurements were performed. Images were magnified by a factor of three (Figs 1, 2), thresholding was employed to enhance the grey-white interface, having first visually identified the interface (Fig. 3). The structures to be measured were outlined manually with a tracker-ball driven cursor (Fig. 4). The area was then automatically calculated by pixel counting. Coronal images could be reformatted in any plane, and manipulated in three dimensions. Reformatting in this fashion allows confirmation of the anterior coordinates of the hippocampus and correlation with the original coronal images to ensure accuracy of measurements. All measurements were performed on original coronal images, rather than reformatted views.

Technique of measurement

Anatomical definition of the posterior limit of the HF was taken as the slice in which the greatest length of fornix was seen. Thresholding was performed using the identify feature, allowing the width and level to be set according to the visually determined grey-white interface in the first image measured (Fig. 3), and these settings used for all subsequent images in a given subject. Mesially, the open end of the hippocampal fissure was taken as the limit of the HF in the posterior and middle portions, the uncal fissure once the intralimbic gyrus was reached in the anterior of the structure. Anteriorly the disarticulation of the hippocampus from the amygdala used the alvear covering of the HF, which enabled distinction. The uncus was included in the measurements, as exclusion generates a source of error. Alveus, fimbria and choroid plexus were all included in the measurements, as exclusion is difficult, and make a trivial contribution overall to volume. Fornix was excluded where identified as a distinct structure posteriorly. It is important to note that using the edge enhancement method, the fimbria and mesial alveus are not outlined as part of the HF, and must be included by visual determination of their margins (Fig. 4). Total areas were then used to calculate the volume, $VH = t.\Sigma aH$, where VH = volume of HF (ml), t = slice thickness (mm), and ΣaH = sum of all hippocampal surface areas (mm^2) (Cavalieri's principle). Amygdala volume was also calculated in a similar manner. Standard anatomical atlases were used to define the structure in coronal sections (Naidich *et al.*, 1987; Duvernoy, 1988; Nieuwenhuys *et al.*, 1988). Calculation of volumes in this fashion requires approximately 30–45 min of operator time. Volumes derived were not corrected for intracranial volume or anterior temporal lobe volumes, as measurement would be time-consuming, and it is not known if HF volumes follow a normal distribution, or are related to total cerebral volume in a linear fashion.

The measurements made in the study were: (i) overall HF and amygdala volumes; (ii) ratio of overall right-left HF volumes (for convenience expressed as smaller to greater); (iii) the hippocampal cross-sectional area in each successive slice was plotted against slice number. This permits morphometric assessment and the identification of focal atrophy within the HF, by slice by slice comparison with that of the contralateral HF. Amygdala measurements were also plotted (Fig. 5). Hippocampal formation volume loss (atrophy) was divided into anterior, posterior or diffuse.

TABLE 2 TEMPORAL LOBE EPILEPSY PATIENTS

Patient sex/age	*Seizure lateralization*	*HF volumes (mm^3) (right/left)*	*HF ratio*	*Morphometry*
1 F/30	LHS	2380/1426	0.60	Asymmetric uniform loss
2. F/36	RHS	2668/3372	0.79	Asymmetric uniform loss
3. F/41	RHS	1992/2188	0.91	Asymmetric uniform RHS volume loss
4. F/38	RHS	2124/2308	0.92	Asymmetric anterior RHS volume loss
5 F/46	RHS	2427/2176	0.90	Asymmetric anterior LHS volume loss
6 M/44	LHS	2335/3922	0.59	Asymmetric uniform loss
7 F/23	LHS	3189/2796	0.88	Asymmetric posterior LHS volume loss
8. F/28	RHS	3247/3725	0.92	Asymmetric anterior RHS volume loss
9 M/18	LHS	2434/3203	0.76	Asymmetric anterior LHS volume loss
10. M/43	LHS	3980/3463	0.87	Asymmetric anterior LHS volume loss
11. F/33	RHS	3981/4492	0.89	Asymmetric anterior RHS volume loss
12. F/34	RHS	3676/4026	0.91	Asymmetric anterior RHS volume loss
13. M/17	LHS	2301/4062	0.57	Asymmetric uniform RHS volume loss
14. M/20	RHS	3055/3846	0.79	Asymmetric uniform RHS volume loss
15 M/41	RHS	3265/3668	0.89	Asymmetric anterior RHS volume loss

Patient sex/age	Seizure lateralization	HF volumes (mm^3) (right/left)	HF ratio	Morphometry
16 M/32	LHS	4130/4347	0.95	Asymmetric anterior LHS volume loss
18 M/44	RHS	4111/3673	0.89	Asymmetric anterior LHS volume loss
19 M/56	LHS	3712/3700	0.86	Asymmetric anterior LHS volume loss
20. M/36	RHS	3970/4401	0.90	Asymmetric anterior RHS volume loss

M = male, F = female, RHS = right-hand side; LHS = left-hand side

Validation studies

Phantom studies, using a cylindrical phantom with defined external and internal measurements, were performed randomly during the study. An identical volume imaging sequence was performed, and data manipulated in the same fashion. Two 5 ml cylindrical cavities filled with water were placed symmetrically inside the phantom, and volume measurements carried out. This demonstrated an accuracy of >99% for linear measurements, and >98% for volumes. Intra-observer consistency was checked by repeated measurements of the same HF on different occasions, 10 times in five patients and five times in 10 patients. Variation was 2–5% for total volume measurements, but <2% for the ratios. Inter-observer variation was established by a neuroradiologist (J.M.S.), blind to all clinical details, on 10 randomly selected subjects. Variation was up to 7% for total volume measurements, but <2% for ratios. The reason for this disparity is that observers choose slightly different margins and landmarks for their areas, but do so symmetrically, giving less variation in the ratio than the total volumes.

RESULTS

Volumes and morphometry in normals

The range of total volumes is listed in Table 1. The ratio of volumes (smaller to larger) in the normal subjects was >0.96 in all cases. The slice by slice HF surface area curves were symmetrical in all cases (as shown in Fig. 5).

Volumes and morphometry in TLE

Total volumes are shown in Table 2. All patients in this group had asymmetric HF volume loss, corresponding to the side of clinical and EEG seizure lateralization, with a ratio of total volumes between 0.59 and 0.95. The slice by slice HF area length curves demonstrated the distribution of the atrophy in all cases. Figure 6 shows a patient with a volume ratio of 0.6, and uniform atrophy. Figure 7 is of a patient with posterior atrophy, and ratio of 0.88. A subject with focal anterior atrophy is shown in Fig. 8. Other patients demonstrated similar striking regional involvement, which sometimes involved only 6–10 mm of total hippocampal length. There was strong correlation between a uniform pattern of atrophy and the occurrence of secondarily generalized seizures in the group of patients with TLE ($P < 0.005$, Fisher's exact test).

TABLE 3 FRONTAL LOBE EPILEPSY PATIENTS

Patient sex/age		*Seizure localization*	*HF volumes (mm^3) (right/left)*	*HF ratio*	*Morphometry*
21	M/29	R mesial frontal glioma	2979/3071	0 97	Symmetrical
22	M/45	R dorsal frontal cortical lesion ?dysplasia	3243/3178	0.98	Symmetrical
23.	M/20	L rolandic Macrogyria	3496/3522	0 99	Symmetrical
24	M/20	L mesial frontal	3541/3506	0.99	Symmetrical
25.	F/43	Bilateral central macrogyria	2466/2569	0 96	Symmetrical
26	F/31	R dorsal frontal focal cortical dysplasia	2388/2308	0 96	Symmetrical
27	M/61	R rolandic. Macrogyria	2998/2968	0.99	Symmetrical
28.	F/19	R rolandic Large destructive hemisphere lesion	2392/2469	0.96	Distorted
29.	F/33	R mesial frontal	3425/3495	0.98	Symmetrical
30	M/41	R orbital frontal lesion, traumatic	3100/3156	0 98	Symmetrical
31	M/33	L mesial frontal	4124/3959	0 96	Symmetrical
32.	F/20	L mesial frontal	2879/2764	0 96	Symmetrical
33	M/36	R mesial frontal	3493/3352	0.96	Symmetrical
34	F/21	R dorsal frontal. Large destructive convexity lesion	3658/3621	0.99	Symmetrical
35.	F/23	R mesial frontal	3410/3342	0 98	Symmetrical
36.	F/27	L rolandic. Bilateral, asymmetric band heterotopia	2727/2659	0 97	Symmetrical
37	F/36	L rolandic	2053/2079	0 99	Symmetrical
38	M/18	R dorsal frontal	3155/3219	0 98	Symmetrical
39	M/22	R frontal polar	3556/3449	0.97	Symmetrical
40	M/30	L mesial frontal	2658/2685	0.99	Symmetrical

M = male, F = female,. R = right; L = left.

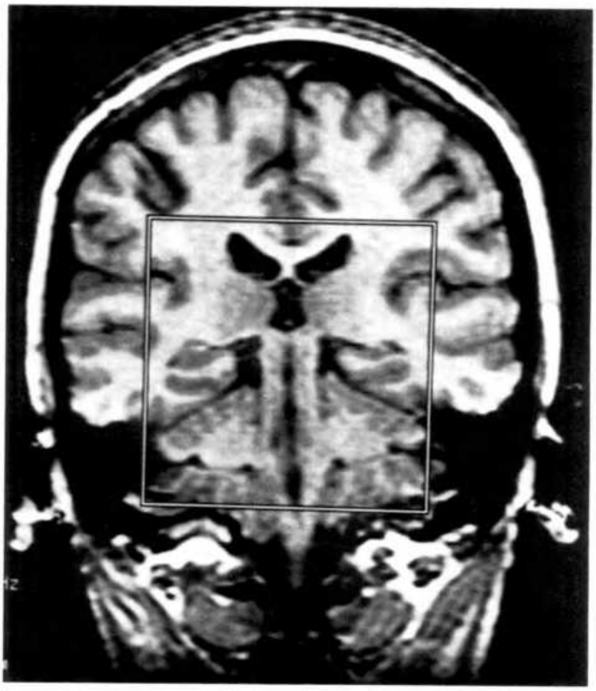

FIG. 1. Coronal MRI (SPGR 35/5/1 TR/TE/NEX) with magnification cursor in place. Patient with TLE: left-sided hippocampal cross-sectional area asymmetry is present.

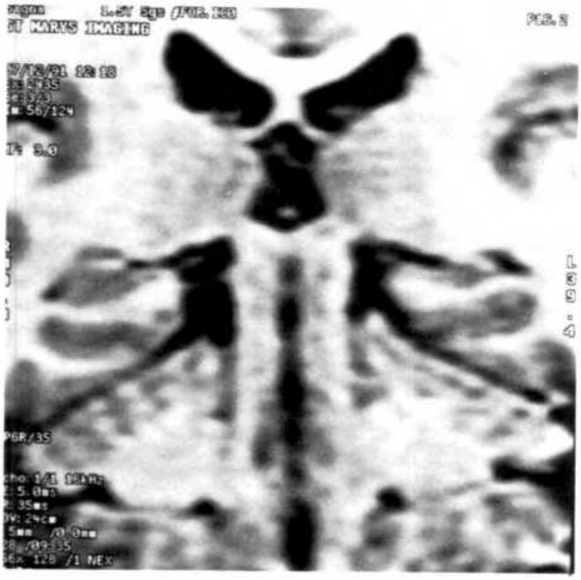

FIG. 2. Magnified region of interest (magnification ×3), for a patient with TLE. Imaging parameters as in Fig. 1.

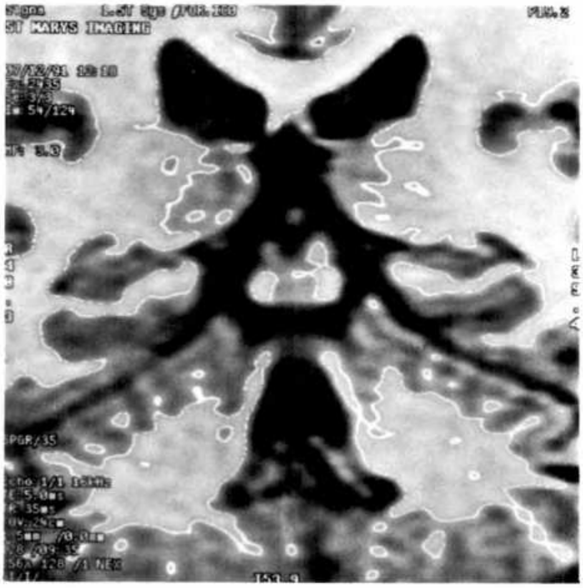

FIG. 3. Thresholding feature applied to magnified image. The grey-white interface is outlined. Note extension of edge into superior hippocampal formation using this feature (*see* text). Imaging parameters as in Fig. 1.

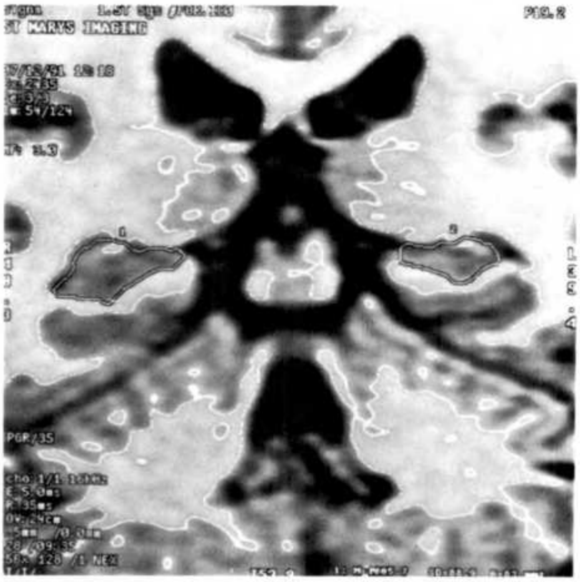

FIG. 4. Hippocampi have been outlined with the cursor, and the area is calculated automatically by pixel counting. Imaging parameters as in Fig. 1.

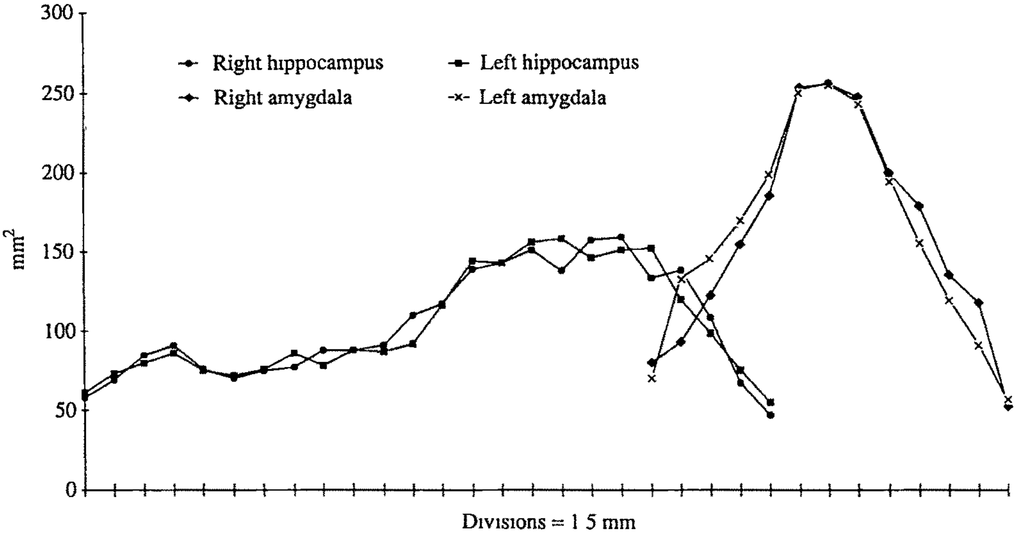

FIG. 5. Slice by slice HF area versus length curve of normal control Both hippocampal formations and both amygdala are shown. The y-axis represents the hippocampal cross-sectional surface area (mm^2) The x-axis is marked in 1 5 mm divisions (slice thickness).

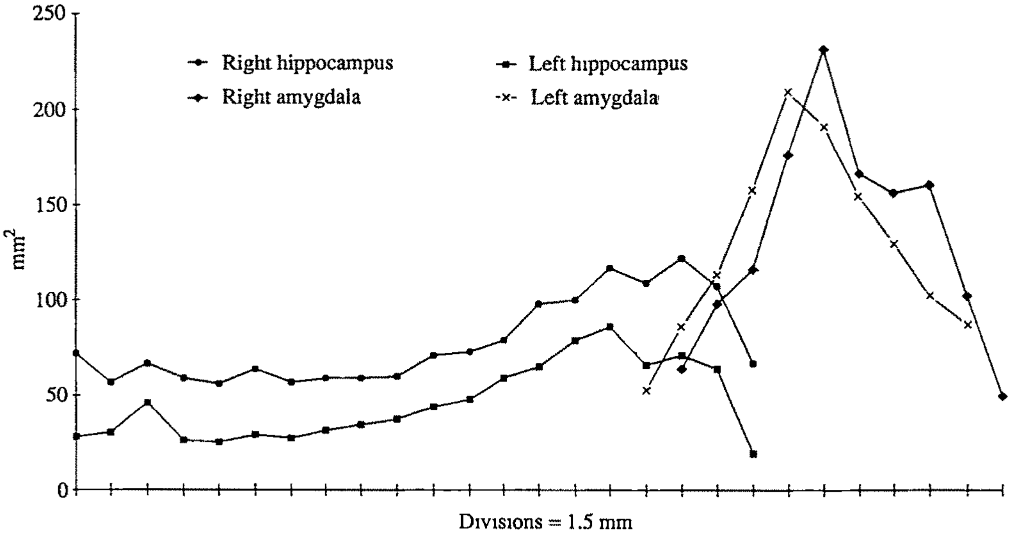

FIG. 6. Slice by slice HF area versus length curve showing widespread volume loss in a patient with TLE. The ratio of hippocampal volumes is 0.59, and the distribution of the changes demonstrated. Amygdala volumes are remarkably symmetrical. The axes are marked as in Fig 5

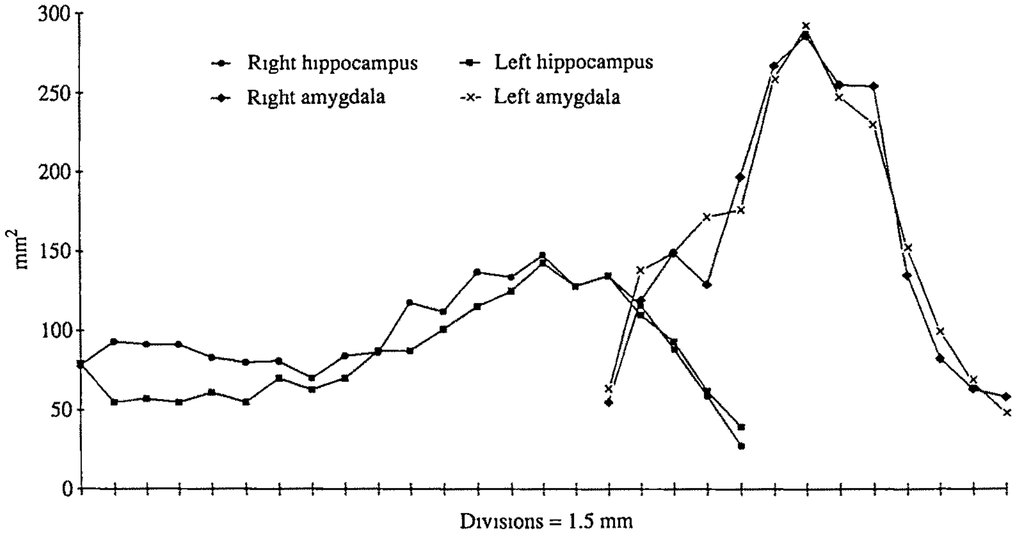

FIG. 7. Slice by slice HF area versus length curve, focal left-sided posterior hippocampal volume loss is revealed in this patient, with a volume ratio of 0.90 The axes are marked as in Fig 5.

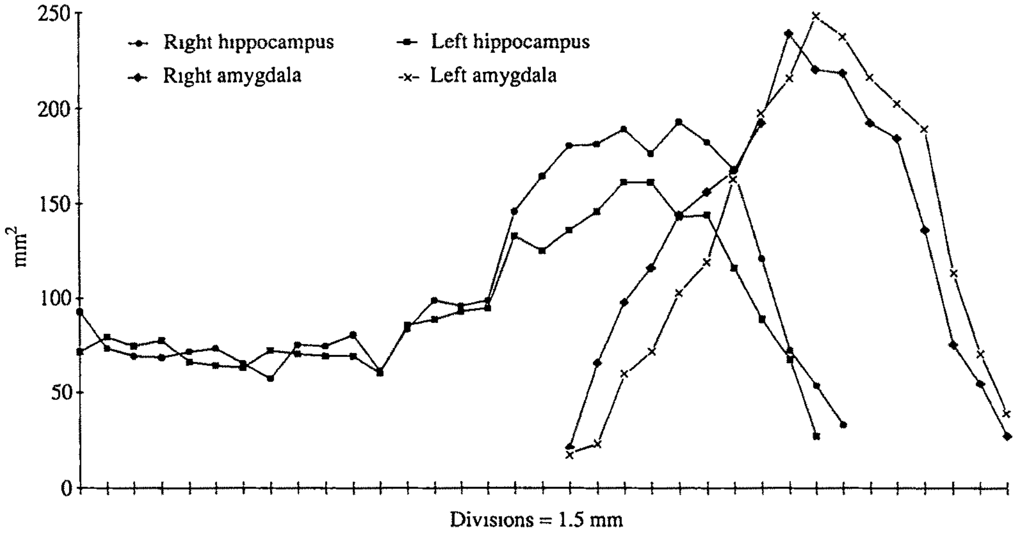

FIG 8. Slice by slice HF area versus length curve, focal left anterior hippocampal volume loss. The volume ratio is 0 86. The axes are marked as in Fig. 5.

Volumes and morphometry in FLE

Total volumes are shown in Table 3. In this group all HF volume ratios were >0.96, as in normal controls. Slice by slice HF area length curves were symmetrical in all cases, except those with large destructive hemisphere lesions where the curves were distorted. With this exception no regional morphometric abnormalities were seen.

DISCUSSION

Volumetric imaging methods have great advantages in epilepsy imaging. Images are detailed enough to perform precise anatomical measurements (Filipek *et al.*, 1989; Vannier *et al.*, 1991), and the spatial resolution is of such quality that high quality reformatting is permitted. This latter capability is of particular importance in situations where an extra-temporal lesion is suspected, as neocortical abnormalities of questionable significance on routine sequences may be analysed and their precise character elucidated. Proton density and T_2-weighted sequences should also be performed. In our study, patient compliance with the short examination was excellent. Image quality can be improved by a greater number of excitations, but this theoretical advantage can be outweighed by image degradation due to movement. Calculation of volumes from coronal information of a structurally anisotropic feature, such as the hippocampus, demands high spatial and intensity characteristic resolution. The spoiled-gradient echo technique employed generates images of sufficiently high signal-noise ratio to be able to make accurate measurements of intensity, enhanced by use of the thresholding techniques described.

Spatial resolution is improved with contiguous fine slices. We feel that this is of critical importance. Previous studies, which have addressed volumetric hippocampal assessment, have been severely restricted by large interslice gaps or thick slices (Jack *et al.*, 1990; Ashtari *et al.*, 1991). Reference to the morphological features of the hippocampus, as depicted in Fig. 5, shows that sampling at a greater interval may result in substantial variation in apparent cross-sectional area in coronal images. Considerable errors may then result if the volume is determined using Cavalieri's principle. It can also be understood how head tilt (left-right) of a relatively minor degree may result in substantial variation in apparent cross-sectional hippocampal area on coronal images. We would urge that all studies, irrespective of intensity resolution characteristics, need to be of fine section if cross-sectional area measurements are used. Anisotropy of the imaged structure and overprojection errors are of considerable consequence when estimating volumes of a small structure such as this. Stereology, the branch of mathematics concerned with the derivation of three-dimensional information from two-dimensional data, allows one to estimate the accuracy of a given sampling frequency for a structure, taking into account degree of anisotropy of the structure. These principles are of great importance in imaging generally, allowing estimation of accuracy of volume measurements generated from a given number of slices (Gundersen, 1986; Cruz-Orive, 1989). Overprojection, when three-dimensional information is projected into a two-dimensional image, is a major problem when dealing with small, anisotropic structures (Roberts *et al.*, 1991). Overprojection may be reduced when imaging the hippocampus by tilting the imaging plane into the oblique coronal plane, but overprojection due to curvature in the axial plane is still not overcome. Using slices of <3 mm thickness is the simplest means of minimizing this source of error. Applying stereological principles (Gundersen, 1986; Gundersen and Jensen, 1987) to this data set shows that for a structure of this size and shape a minimum of 10–12 slices in the coronal plane are required for a coefficient of error of <5%, which we believe necessary to distinguish involvement. This number of slices corresponds to approximately 3 mm sampling intervals in the coronal plane. The coefficient of error for a sample set of six slices (approximately 5 mm thick sections), as has been presented in other series, allows an accuracy of only 80−90%, presuming overprojection is negligible. For the usual hippocampal total volume of 3−4 ml, then at least 0.3−0.4 ml error is inevitable with this slice thickness. Given that the volume loss is often of this order of magnitude in subjects with TLE, this slice thickness is therefore inadequate. The minimum sampling interval should be no greater than 3 mm in volumetric studies of the hippocampus. Increasing slice thickness will increase errors due to overprojection in the images, and so even if the structure is sampled at 3 mm intervals, slices as thin as possible should be used if the volume measurements are to be accurate.

Disarticulation of the anterior hippocampus from the amygdala is the most difficult part of these measurements, and the most prone to error. One study (Ashtari *et al.*, 1991) defined the mammillary body as the anterior extent, and excluded the anterior pole of the hippocampus where distinction from the amygdala is difficult. We were able to reliably distinguish these structures, due to the fine slices and excellent T_1 signal characteristics provided by the SPGR. High quality reformatting assists in this process, as the anterior limit may be defined and correlated with the coronal slices. Distinguishing these structures is important, as the pathology may lie exclusively in this region. The time taken to perform the measurements manually is not restrictive; an experienced observer is able to complete a study in 30−45 min in a standard case.

Other features suggestive of hippocampal sclerosis on T_2-weighted images are increased signal intensity and loss of internal architecture (Kuzniecky *et al.*, 1987; Jackson *et al.*, 1990). It is not known to what degree the volumetric abnormalities correspond to the signal changes, but their localized nature in some cases suggests they may be overlooked with an examination using larger slices and interslice gaps. Disruption of internal architecture was not a prominent feature of our series, even when volume loss was severe the usual anatomical features could usually be distinguished, albeit shrunken. We did not acquire T_2-weighted coronal images for comparison, but none of our TLE group with volume loss had signal change in the T_2 axial images. Detection of abnormalities in this plane could have been improved by orienting the axial images into the oblique plane, as demonstrated in other studies (Jackson *et al.*, 1990). Quantitative studies of T_2 characteristics may also increase the sensitivity of these studies. We did not perform our coronal images in an oblique plane, as the contribution of partial voluming at this slice thickness is negligible.

A number of striking findings are made in our study. First, all the TLE group showed HF volume ratios of <0.96, and none of our controls or FLE cases. In our group of patients, this method was highly effective at differentiating between TLE and FLE. We stress, however, that the patient groups were well-defined temporal or frontal cases with unequivocal clinical features, and well-lateralized EEG findings. It is quite possible that this type of study would not allow absolute distinction in patients with less clear-cut seizure syndromes. Furthermore, none of our patients with TLE has yet undergone resection, and our results should be interpreted with caution in the absence of pathological correlation, although previous studies have shown a correlation between hippocampal sclerosis and volume changes (Bronen *et al.*, 1991; Cascino *et al.*, 1991). Nevertheless, differentiation of frontal and temporal seizure patterns is often difficult clinically, and the findings of this study demonstrate the great potential value of volumetric analysis not only in lateralizing temporal lobe disease and localizing atrophy within the HF, but also helping distinguish frontal and temporal epilepsies.

Another important aspect of our method is the ability to demonstrate the position within the HF of focal volume loss (atrophy). No previous study has demonstrated this, but it is of great clinical interest. The distribution of the atrophy within the HF may be assessed using the slice by slice HF area-length curves, and we have found patients with focal atrophy of 6 – 10 mm length. It is possible, therefore, to differentiate patients with anterior, posterior or widespread volume loss (Figs 6 – 8). Patients with generalized seizures were more likely to have widespread volume loss, suggesting the possibility of correlation of clinical seizure characteristics with the distribution and extent of MRI determined volume loss. Our study does not include enough patients to make definite statements regarding these aspects, and it is unlikely in such a complex situation as seizure elaboration that any simple relationship will exist. The posterior extent of lesions has obvious significance in planning surgery, as prognosis has been demonstrated to be related to the posterior extent of lesion histologically (Babb and Brown, 1987), as well as to the extent of mesiobasal resection (Nayel *et al.*, 1991).

None of the patients with FLE showed markedly decreased overall volumes, nor was focal atrophy observed on slice by slice HF area length curves. However, few of the patients with FLE had complex limbic symptomatology, and such patients may be more liable to have sustained secondary hippocampal damage. None of the patients showed psychological evidence of more widespread cerebral damage, and again, this group may be more likely to have hippocampal involvement. It is possible that FLE causes symmetrical bilateral volume loss, and as we compared right and left HF, this would obscure ratio and morphometric changes. Given the range of volumes and close correlation of morphometric studies between normals and FLE patients, this is, however, unlikely.

The problem of bilateral hippocampal pathology is not easily addressed by volumetric studies, particularly when results are expressed as ratios. Many studies have demonstrated frequent bilateral asymmetric damage, perhaps as frequently as 80% of cases of TLE (Sano and Malamud, 1953; Margerison and Corsellis, 1966; Mouritzen-Dam, 1982). Bilateral symmetric damage is less frequent, probably occurring in < 10% of cases (Babb and Brown, 1987). We have selected cases of TLE well lateralized on clinical and EEG criteria for this study, but the problem of detecting small degrees of bilateral damage remains. Expression of hippocampal volume distribution may help in this regard, enabling comparison between the normal contour and that of patients with focal damage. This may make clear bilateral volume changes in patients with gross asymmetries.

Finally, in the normal group, we observed no significant overall HF volume difference and no focal volume loss. Left-right differences in hippocampal volume have been contradictory in previous studies (Jack *et al.*, 1988, 1989; Ashtari *et al.*, 1991). We did not study left-handed subjects, but no significant left-right difference in HF volumes was found in normals. Our study used much finer slices, and this may account for the difference observed, as less frequent sampling of a structure of this size and shape will significantly increase error. We believe the rigorous application of stereological principles to this kind of volume estimate is essential to avoid errors of this nature. Volumetric

MRI is uniquely suited to the application of these principles. Exclusion of the uncus (Jack *et al.*, 1989) may contribute to this difference, though this is unlikely to explain the differences in these studies. Similar estimates of symmetry were made in normals in another study (Ashtari *et al.*, 1991), and this study used 3.1 mm thick slices. The range of normal HF volumes in this study (2595–3700 mm^3) is less than in the study which published total volumes (Ashtari *et al.*, 1991), where the range was 1710–3705 mm^3. The range of normal volumes is obviously great, and we were unable to demonstrate a simple linear relationship of this volume to total cerebral volume.

This study has demonstrated the value of HF volumetrics in distinguishing frontal and temporal epilepsies in a group of well-defined patients, and provides unique information regarding the distribution of these changes. Work is in progress to establish the sensitivity and specificity of these changes in a larger group of patients with temporal lobe and extra-temporal epilepsies. Thus, volumetric MRI techniques show great promise in providing detailed *in vivo* anatomical measurements of clear clinical importance. We consider the method described in this paper to be superior to all others in this regard.

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MOVEMENT-RELATED POTENTIALS RECORDED FROM SUPPLEMENTARY MOTOR AREA AND PRIMARY MOTOR AREA

ROLE OF SUPPLEMENTARY MOTOR AREA IN VOLUNTARY MOVEMENTS

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SUMMARY

Movement-related potentials (MRPs) were recorded from subdural electrodes chronically implanted in the interhemispheric fissure in two patients being evaluated for epilepsy surgery. Different types of movements (finger, foot, tongue and vocalization) were executed. Foot movements elicited a clearly defined, well-localized slow negativity or positivity (Bereitschaftspotential, BP) preceding electromyogram (EMG) onset. These BPs were seen from the contralateral primary motor foot area and also from bilateral supplementary motor areas (SMAs) with equivalent amplitudes and temporal evolutions. A steeper potential [negative slope (NS′)] occurred about 300 ms before EMG onset and the motor potential (MP) started 100 ms before EMG onset. Negative slopes and MPs also arose from the contralateral primary motor area as well as from the bilateral SMAs. Finger movements elicited well-localized BPs and NS′ which were generated from the bilateral SMAs, but were of higher amplitude on the contralateral SMA. Motor potentials started 50 ms prior to EMG onset and arose exclusively from the contralateral SMA. Tongue protrusions and vocalizations also elicited BP, NS′ and MP which were seen in the bilateral SMAs.

Movement-related potentials for different types of movements had a somatotopic distribution in the SMA, which was consistent with the SMA somatotopic organization defined by electrical simulation. Movement-related potentials for tongue movements and vocalization had a similar distribution and waveform. It was concluded that bilateral SMAs generate well-defined MRPs consistent with the assumption that the SMA plays a significant role in the organization of voluntary movements. However, the MRPs from the bilateral SMAs do not have characteristics which are different from those of the primary motor area. This suggests the hypothesis of 'supplementary' function for SMA, and does not support the hypothesis of 'supramotor' function.

INTRODUCTION

Cortical potentials associated with voluntary movements are identified as movement-related potentials (MRPs). Extensive studies have been carried out to clarify the actual cortical generators and neuronal mechanisms involved in their generation, hoping that it could shed some light on our understanding of the neuronal mechanisms involved in

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motivation, preparation, initiation and control of voluntary movements. Scalp recordings (Kornhuber and Deecke, 1965; Vaughan *et al.*, 1968; Deecke *et al.*, 1969; Shibasaki *et al.*, 1980; Barrett *et al.*, 1985) suggested that not only the primary motor area but also the supplementary motor area (SMA) participated in the generation of MRPs, especially the early component, usually known as the Bereitschaftspotential (BP) (Boschert *et al.*, 1983; Boschert and Deecke, 1986; Deecke, 1990), because it is always symmetrically distributed with the maximum at the vertex. However, this distribution could be explained by a summation of two potentials arising from the bilateral primary motor areas equally (Neshige *et al.*, 1988). Experimental studies in animals with subdural and depth electrodes, microstimulation and unit recording techniques have suggested the generation of activities preceding the movement onset from bilateral primary motor areas as well as SMA (Arrezo and Vaughan, 1975, 1980; Hashimoto *et al.*, 1979; Sasaki *et al.*, 1979, 1981; Tanji and Kurata, 1982, 1985; Kurata and Tanji, 1985). However, there are few intracranial studies in humans (Lee *et al.*, 1986), and previously only one study reliably showed that bilateral primary motor areas were the main BP generators and moreover, that the potentials generated by SMA were of lower amplitude and seemed to start later than the BPs in the primary motor area started (Neshige *et al.*, 1988). Although other authors have stated that the SMA is a supramotor area for programming and execution of voluntary movements (Orgogozo and Larsen, 1979; Roland *et al.*, 1980; Goldberg, 1985), the generator sources of MRPs have not been satisfactorily identified, and from an electrophysiological point of view, the role of the SMA in human remains unclear.

On the other hand, previous MRP studies have focused exclusively on finger or hand movements; whereas generator analysis of MRPs associated with foot movements is still inconclusive because the primary foot motor area is on the mesial brain surface where potentials are inaccessible to scalp electrodes (Vaughan *et al.*, 1968; Shibasaki *et al.*, 1981; Brunia and Bosch, 1984), and no invasive studies have been carried out for foot movements.

In the present study we have been able to critically examine these questions about MRPs, in the unique opportunity presented by recording from electrodes chronically implanted in the interhemispheric fissure of patients with medically intractable supplementary motor type partial seizures.

MATERIALS AND METHODS

Materials

Two epileptic patients with medically intractable supplementary motor-type partial seizures (Morris *et al.*, 1988), who were evaluated for surgery for epilepsy using chronically implanted subdural electrodes according to the Cleveland Clinic Epilepsy Surgery Protocol (Engel, 1987), were studied. The interhemispheric arrays consisted of two identical sheets of silicon rubber containing a 2×5 set of electrodes which were glued together back to back so that the recording surface of each set of 10 electrodes faced in opposite directions (Figs 1, 2). After placing these bidirectional plates in the interhemispheric fissure, one set of 2×5 electrodes recorded from the mesial region subdurally, and the other set recorded from the contralateral mesial region epidurally (Figs 1, 2). Each electrode was 3 mm in diameter and the centre-to-centre interelectrode distance was 1 cm. Recording invasively with subdural electrodes can (i) help to identify the extent of the epileptogenic focus by recording ictal as well as interictal activity, and (ii) assess the function of the cortex around the epileptogenic focus through stimulation. This information is essential for rational planning of the surgical resection (Lueders *et al.*, 1982; Lüders *et al.*, 1987). A more detailed description of the methodology

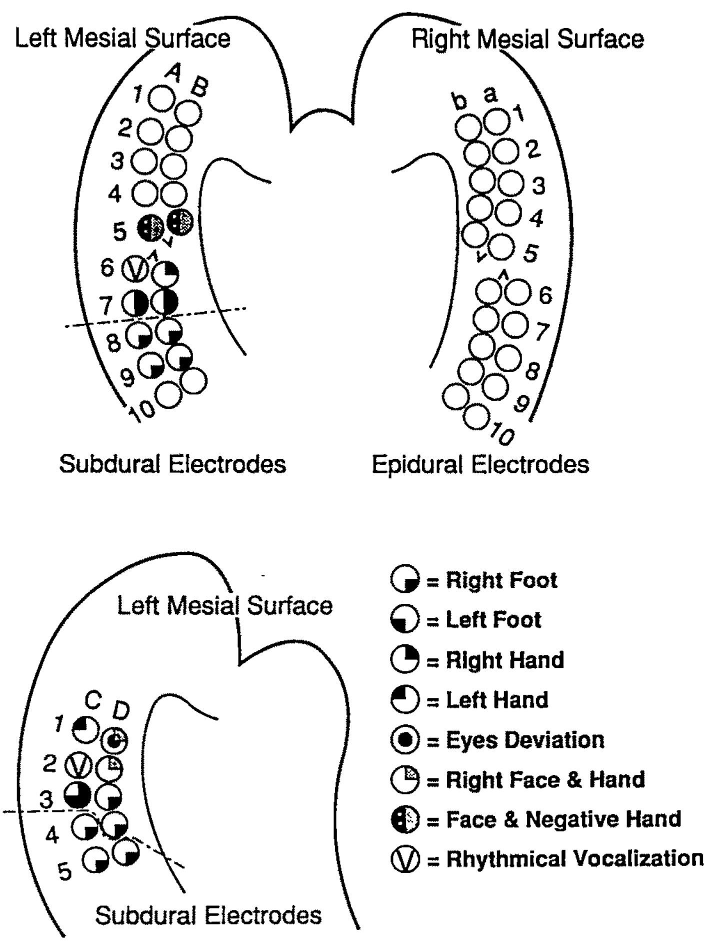

FIG. 1. Schematic representation of the placement of interhemispheric plate electrodes in patients 1 (*top*) and 2 (*bottom*) Cortical stimulation was done only on the subdurally placed electrodes (*left side*). The diagrams shown in this figure were copied directly from the lateral X-ray film as well as the sagittal views of the MRI. The broken lines show the boundary between the primary motor foot area posteriorly and the SMA anteriorly. Both patients had also another 8×5 subdural electrode plate over the left frontal convexity, which is not shown here. The site of the responses to electrical stimulation of each of the different implanted electrodes in the array is indicated by the symbols in the figure. More details are given in the text.

used to evaluate epileptic patients with subdural plates is available elsewhere (Hahn and Lüders, 1987). Informed consent was obtained from all patients following procedures approved by the Institutional Review Board at the Cleveland Clinic Foundation.

Patients

Patient 1 was a 29-yr-old right-handed man with a history of intractable seizures since the age of 23 yrs. The aetiology was unknown, and the past medical history was normal. His seizure type was consistent with supplementary motor seizure and the attacks usually occurred at night. They consisted of sudden arousal followed by whole body stiffening with abrupt posturing of both arms in abduction more pronounced

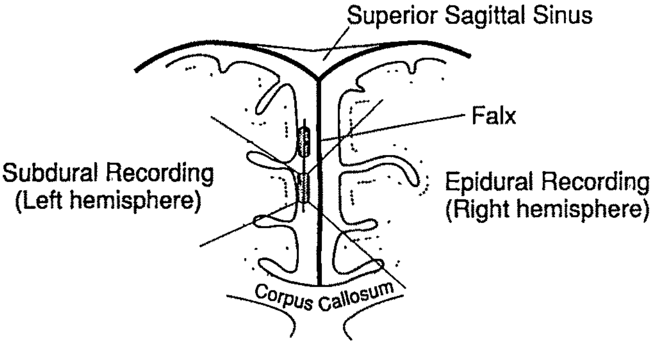
Superior Sagittal Sinus
Falx
Subdural Recording (Left hemisphere)
Epidural Recording (Right hemisphere)
Corpus Callosum

FIG. 2. Schematic representation of the coronal view of interhemispheric plate electrodes. The plate consisted of two arrays of 2×5 electrodes mounted on silicone rubber sheets, which were attached to each other and faced in opposite directions. When the bifacial plate was placed in the left-sided interhemispheric fissure, the left mesial brain potentials were recorded subdurally, and the right mesial brain potentials were recorded epidurally.

on the right side. This was followed by screaming, thrashing and writhing lasting less than 1 min. There was little or no postictal confusion. The seizure frequency was 5–7/day in spite of intensive medical treatment with high doses of phenytoin, phenobarbital, carbamazepine and valproic acid either as monotherapy or in combination. His neurological examination was normal. Magnetic resonance imaging (MRI) of the brain with gadolinium enhancement was normal. The intracarotid sodium amobarbital (Wada) test demonstrated speech dominance in the left hemisphere and bilateral memory representation (Rausch, 1987). The Wechsler Adult Intelligence Scale (WAIS) full-scale intelligent quotient (IQ) was 79, with a verbal IQ of 78, and a performance IQ of 88. A noninvasive video/electroencephalogram (EEG) seizure monitoring with scalp electrodes revealed that the seizures were associated with centrally located bursts of ictal sharp transients. No epileptiform interictal activity was noticed.Two 2×5 subdural plates of the type described above were implanted in the left-sided frontocentral interhemispheric fissure anteroposteriorly (Fig. 1) as well as an 8×5 subdural plate over the left frontal lateral convexity (not shown in the figure). The subdural plates were implanted for 2 wks. Seizure monitoring, evoked potential studies and cortical stimulation studies were carried out to localize the epileptogenic zone more accurately and to define the function of the adjacent cortex. After completing these evaluations, the subdural plates were taken out and the epileptogenic zone was resected sparing the adjacent functional areas. The pathological diagnosis of the resected specimen was cortical heterotopia. Postsurgically, no neurological deficits were seen and the patient has been seizure free

Patient 2 was a 17-yr-old right-handed women with medically intractable seizures since the age of 7 yrs. She was the product of a normal pregnancy and delivery. Developmental milestones were normal and past history was unremarkable. Her seizures consisted of sudden onset of backward falling with bilaterally tonically abducted arms and eye deviation to the right. Consciousness was not altered during the seizures. Initially the seizures occurred once a month, but in the past 4 yrs they have increased to 2–5/day. Her neurological examination was unremarkable. An MRI of the brain demonstrated an abnormal area of increased signal on T_2-weighted and spin-density images 2.5 cm in diameter in the left frontocentral region at the grey-white matter junction in the high lateral convexity. A mass effect was not seen and the size of the lesion has not changed in the last 5 yrs. Wada test demonstrated speech dominance in the left hemisphere and bilateral memory representation. The Wechsler Adult Intelligence Scale full-scale IQ was 104, with a verbal IQ of 98, and a performance IQ of 110 The patient has previously been on phenytoin, phenobarbital, primidone, carbamazepine and valproic acid either as monotherapy or in combination with 'therapeutic' or 'supratherapeutic' blood levels, and in the last year the patient has been on carbamazepine (2300 mg/day) and chlorazepate (22.5 mg/day). However, her seizures were poorly controlled. A noninvasive video/EEG monitoring showed that her supplementary motor seizures were associated with ictal as well as interictal activities arising from the vertex region. The patient underwent an invasive video/EEG monitoring with a 2×5 subdural plate at the central left-sided interhemispheric fissure (Fig. 1) and an 8×5 subdural plate (not shown in the figure) at the left frontal high convexity. After seizure monitoring, evoked potential studies and cortical stimulation studies were carried out for 2 wks, the plates were removed, and the epileptogenic zone including the lesion was resected sparing the adjacent functional areas. The pathological diagnosis of the lesion was ganglioglioma. After resection, no neurological deficits were seen and the patient had two brief fragmented seizures lasting only several seconds in the first week. The patient has been seizure free since then.

Cortical stimulation and somatosensory evoked potentials

Cortical stimulation was carried out by applying electrical current to each electrode at progressively increasing intensity until (i) either movements or paraesthesiae were elicited, or (ii) after-discharges occurred, or (iii) a maximum stimulus intensity of 15 mA was reached. Electrodes at which no movements or paraesthesiae occurred were tested further for interference with ongoing movements or other higher cortical functions at a stimulus 0.5 mA below the after-discharge threshold, or at 15 mA if no after-discharges were seen at 15 mA. The stimulus consisted of 5 s trains of 0.3 ms duration alternating polarity square wave pulses, delivered at 50 Hz. Brain regions, at which stimulation elicited muscle contraction, were identified as positive motor, and cortical areas at which there was interference with tonic motor activity or with rapid alternating movements were identified as negative motor (Lüders *et al.*, 1985). Two or more cortical functions could be seen occasionally at the same electrode. Because of the relatively large size of the electrodes, they could overlay two adjacent cortical functional areas at the same time.

On the mesial surface, the SMA was identified by its unique responses as described by Penfield and Welch (1951). Responses consisted of predominantly tonic positive motor responses of the upper as well as lower limb and of the trunk, neck and facial areas, occurring unilaterally or bilaterally. Stimulation of the SMA also elicited negative motor response affecting all parts of the body

Cortical potentials evoked by electrical stimulation of the right posterior tibial nerve at the ankle were recorded from the same subdural electrodes using the methods described in detail elsewhere (Lesser *et al.*, 1987).

Recording techniques of MRPs

Cortical recordings were made simultaneously from 20 to 40 intracranial electrodes in both patients during voluntary movements consisting of finger movements, foot movements, tongue protrusions and vocalizations. Electromyogram (EMG) recordings were made from a pair of cup electrodes placed over the skin overlying the extensor muscle of the middle finger in the forearm for finger extensions, and the anterior tibialis muscle in the shin for ankle extensions. For tongue protrusions and vocalizations, the glossokinetic potential (GKP) was recorded with an electrode over the skin at the upper edge of the nasolabial fold on either side. The EMG of the orbicularis oris muscle was also monitored with a pair of electrodes 3 cm apart around the mouth. For vocalizations, the phonogram was recorded with a microphone placed 5 cm in front of the patient's mouth. The electro-oculogram (EOG) was recorded from an electrode placed 1.5 cm below the right outer canthus.

All the recordings were performed at the Epilepsy Monitoring Unit simultaneously with continuous video/EEG seizure monitoring. In patient 1, right and left middle finger extension, right and left ankle extension and tongue protrusion tasks were performed on three different days. In patient 2, right and left ankle extension, tongue protrusion and vocalization tasks were performed on two different days. The subjects laid on a bed or were seated comfortably in a reclining chair. For finger movements, brisk voluntary extensions of the middle finger at the metacarpophalangeal joint of one hand, followed by a return to the resting position by relaxing the extended finger were used as the trigger movements. For foot movements, the patient was seated and both heels were placed on a soft pad on the floor, with hip and knee joints semi-flexed and ankle joints held in a natural flexed position. Brisk extensions of an ankle joint were used as the trigger movements. For tongue protrusions, the jaws were kept relaxed in a slightly opened position Brisk forward tongue protrusions followed by a return to the resting position by relaxing the protruded tongue were used as the trigger movements. For vocalizations, the patient was kept relaxed with his/her mouth closed loosely. Explosive vocalizations of the word 'pine' were used as the trigger events. For all tasks, each movement was repeated voluntarily without any external cue signal, at self-paced irregular intervals of more than 5 s. The subjects were told to remain quiet, especially avoiding eye movements. They were also asked to avoid face, tongue and mouth movements for tongue protrusion and vocalization tasks. The subjects were asked to postpone the next trigger movement for several seconds if accidental movements occurred prior to the trigger movement. The subjects were also instructed not to count the trigger movements. A training period was given before the trial task, and the training continued until the examiner was satisfied that the subject consistently produced brisk trigger movements preceded and followed by complete relaxation. A recording session typically lasted 5–6 min and was repeated on two or three occasions for each kind of task, separated by a break of a few minutes.

All electrodes were connected to two 32-channel Grass model 12 (EEG amplifiers) and an 18-channel Grass model 7 (EEG machine) for amplification, filtering and simultaneous monitoring. All subdural electrodes, the EOG electrode, and the GKP electrode were referenced to one of the subdural electrodes over the lateral convexity. The reference electrode was located between the anterior and middle one-third of the high frontal convexity to minimize GKP and EOG artefacts. Among these electrodes an electrode was chosen which on cortical stimulation did not elicit any symptom or sign, and for which electroencephalography showed no ictal or interictal epileptiform discharges. Sensitivity for the subdural cortical recording was 70 μV/mm, and for the EOG, GKP, EMG and phonogram it was 7 μV/mm. The low frequency filter (LFF) was set to 0.01 Hz for cortical and EOG recordings, and to 10 Hz for EMG and phonogram recordings. The high frequency filter (HFF) was set to 100 Hz for cortical and EOG recordings, and to 10k Hz for EMG and phonogram recordings. The GKP was recorded with two different filter settings: i.e. identical to both the cortical as well as EMG filter settings in order to monitor for preceding slow GKP artefacts mimicking brain potentials. A 60 Hz notch filter was used on all channels. Either paper recordings at a paper speed of 15 mm/s, or signal monitoring with a graphic terminal at an equivalent paper speed of 30 mm/s were utilized throughout the entire examination.

These electrographic output signals were collected and stored on a PDP 11/44 (DEC) computer which digitized each channel at 200 samples per second for subsequent off-line analysis. The digitized data from each trial were stored continuously for up to 85 min on a removable computer disk.

Data analysis

The stored EEG signals were averaged time-locked to the onset of the trigger movements with special computer programs (Neshige *et al.*, 1988) which represent modifications of the methods originally described by Barrett *et al.* (1985). For finger and foot movements, the EMG onset of the trigger movement was visually identified on the computer screen terminal and used as the time-locking fiducial Visual inspection of each trial and on a graphic terminal also permitted editing to obtain essentially artefact-free averages of MRPs. Movements which were not brisk enough to identify a clear EMG onset or movements associated with other recording artefacts were eliminated. For tongue protrusion and vocalization tasks, GKPs recorded at EMG filter settings were used as time-locking fiducials. If there was preceding muscle activity of the mouth and/or tongue and if GKP activity recorded at EEG filter settings preceded GKP activity recorded at EMG filter settings, the trials were eliminated. Trials in which the phonogram did not become active at the time of or just after GKP onset for vocalization were also eliminated.

All signals were averaged 2000 ms before to 1000 ms after the time-locking trigger fiducial point. A total of 150–300 trials were averaged for each task. Two subensemble EEG averages of 75–150 trials were made. Computer-assisted methods were used to measure all time intervals and amplitudes. Amplitude measurements were based on callibration data collected immediately preceding data collection. The baseline for each channel was determined from the averages of the first 400 ms of the recording epoch, i.e. 2000–1600 ms before the fiducial point.

RESULTS

Cortical mapping (Fig. 1)

Cortical functional mapping of the mesial brain surface was based on the results of electrical stimulation and on the distributions of evoked potential responses to the right posterior tibial nerve stimulation. The site of the responses to electrical stimulation through each of the different implated electrodes is indicated by the symbols in Fig. 1. Only the subdurally implanted side (left side, in both patients) of the bifacial interhemispheric plates was stimulated because electrical stimulation of epidurally implanted electrodes, even at low stimulus intensity, elicited pain most probably arising from the falx cerebri (Lesser *et al.*, 1985) (Fig. 2).

In patient 1, stimulation at A8, B8, A9 and B9 elicited twitching (positive motor response) of the right lower extremity. Stimulation of A7 and B7 elicited simultaneous tonic abductive posturing of the right upper and lower extremities, and stimulation of B6 produced tonic posturing of the right upper extremity. Rhythmically interrupted vocalization was produced by stimulation of A6 corresponding most probably to the positive motor area of vocal cords, larynx, pharynx and tongue muscles (Penfield and Welch, 1951). Stimulation of A5 and B5 elicited a positive motor response of the tongue and neck and a negative motor effect of the fingers bilaterally (Lüders *et al.*, 1988). Stimulation of the right posterior tibial nerve elicited a positivity at 40.2 ms (P37) and a negativity at 45.9 ms (N45), which was maximum at A8, B8, A9 and B9. These data suggest that A5, B5, A6, B6, A7 and B7 correspond to the SMA, and that A8, B8, A9 and B9 were located over the primary motor foot area. No symptoms were produced when stimulating A1, B1, A2, B2, A3, B3, A4, B4, A10 and B10.

In patient 2, stimulation elicited responses at all subdural electrodes placed on the left side. Stimulation at C4, C5 and D5 elicited clonic movements of the right ankle and hip consistent with stimulation of the primary motor area. Stimulation at D3 and D4 elicited slow posturing with tonic right hip abduction and inversion of the right ankle, characteristic of stimulation of the SMA as originally described by Penfield and Welch (1951). Stimulation at C3 elicited a positive motor response of both lower extremities as well as of the right upper extremity. Stimulation at C2 elicited rhythmically interrupted vocalization. Positive motor responses of the right face and right upper extremity were elicited by stimulation at D1 and D2. In addition, version of the eyes and head to the right also occurred when stimulating D1. Stimulation at C1 produced a positive motor response in the left ipsilateral upper extremity. No negative motor responses were seen. The highest amplitude potentials to stimulation of the right posterior tibial nerve occurred at C4, C5 and D5 with a positivity at 39.4 ms (P37) and a negativity at 45.1 ms (N45). From these data it was concluded that C1, D1, C2, D2, C3, D3 and D4 were located over the SMA, and that C4, C5 and D5 were over the primary motor foot area.

Movement-related potentials associated with finger movements (Figs 3, 4, 5)

Figure 3 shows MRPs associated with right middle finger extension in patient 1. The left half of Fig. 5 highlights the waveforms at A5, B5, A6, B6, A7 and A8. The MRPs on the left mesial surface were recorded subdurally and on the right mesial surface epidurally (Fig. 2). A clearly defined negative slow potential preceding EMG onset (Bereitschaftspotential, BP) (Kornhuber and Deecke, 1965), was seen at only one electrode (B6). This electrode corresponded to the positive motor region of the right upper extremity in the SMA. The BP started about 600 ms prior to EMG onset, and its slope became steeper about 400 ms before EMG onset [negative slope (NS′)] (Shibasaki *et al.*, 1980). At 50 ms before EMG onset this potential had an amplitude of -30 μV, after which the rate of slope increased dramatically (motor potential, MP) (Deecke *et al.*, 1969). The MP peaked 65 ms after EMG onset with an amplitude of -46.3 μV, and then slowly decayed over the following 1 s. The temporal evolution of these potentials

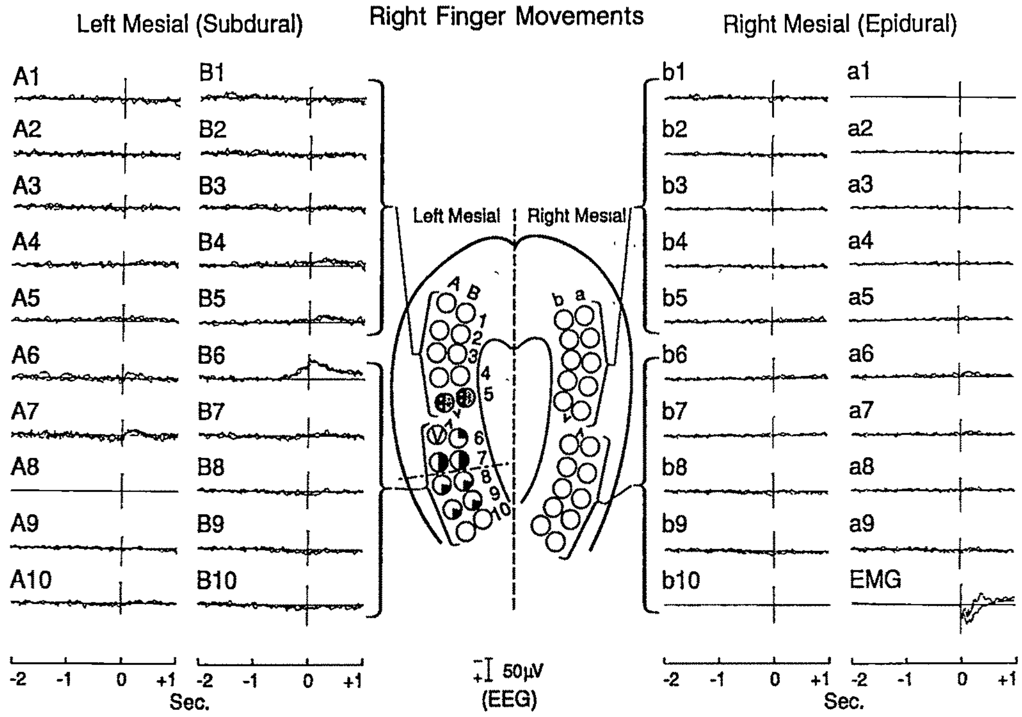

FIG. 3. Movement-related potentials associated with voluntary, self-paced right middle finger extensions recorded from the left mesial brain subdurally and from the right mesial brain epidurally in patient 1 Clearly defined, extremely localized potentials were seen from only B6. EMG = electromyogram, EEG = electroencephalogram.

was similar to the description of MRPs in the primary hand motor area by Neshige *et al.* (1988). In addition, a small positive potential preceding EMG onset was seen at A7 which was located 1.4 cm away from B6. This potential started about 700 ms prior to EMG onset and its slope remained constant until EMG onset, i.e. without appearance of a NS′. At EMG onset a steep negative potential occurred and peaked 200 ms later. The onset and peak time of this potential were slightly later than those of the MP at B6 (by 50 ms and 135 ms, respectively). Similar small negative potentials which started at EMG onset were also seen at A6, B4 and B5. The other electrodes on the left mesial surface and all electrodes from the right mesial surface were silent throughout.

Figure 4 shows MRPs associated with left middle finger extension in patient 1, and the right half of Fig. 5 highlights the waveforms at A5, B5, A6, B6, A7 and B7. A clearly defined negative BP preceding EMG onset was seen only at B6. The BP started about 500 ms prior to EMG onset and a NS′ became evident about 400 ms before EMG onset. The amplitude of NS′ at 50 ms before EMG onset was -20 µV. This potential rose slightly to a peak amplitude at EMG onset of -27 µV. No following MP was seen. Both the BP and the NS′ associated with left finger movement were about 60% smaller than those with right finger movement. A small positive potential preceding EMG onset ('positive BP') was also seen at A7 starting about 1100 ms prior to EMG onset. Small negative potentials were also seen from the epidural recording of the right mesial surface

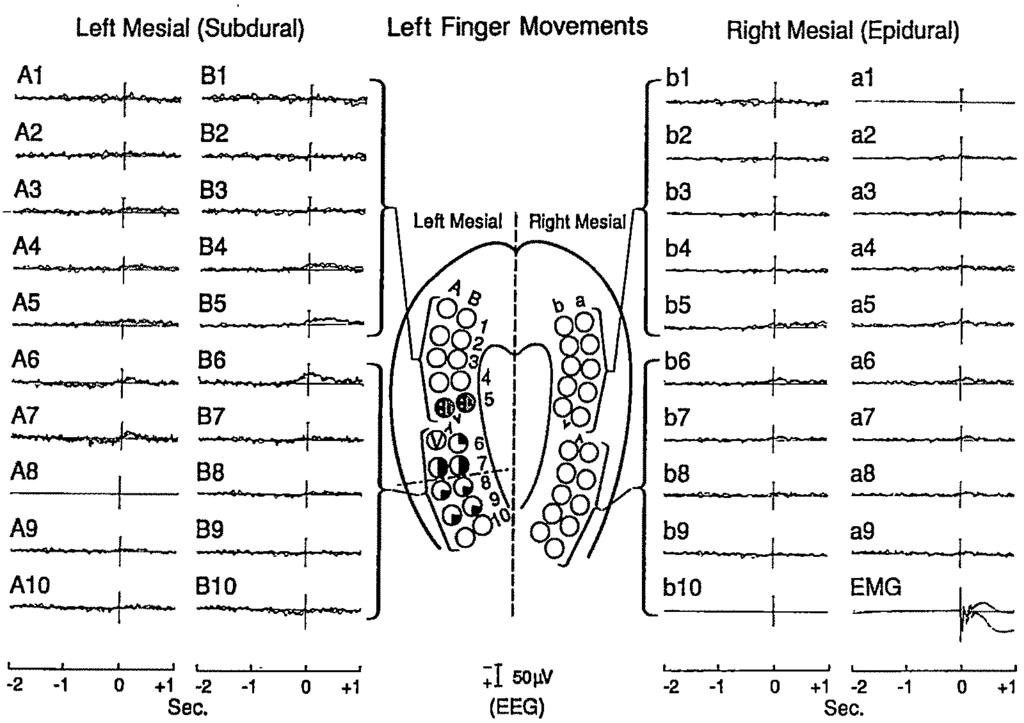

FIG. 4 Movement-related potentials associated with voluntary, self-paced left middle finger extensions recorded from the left mesial brain subdurally and from the right mesial brain epidurally in patients 1. Clearly defined, extremely localized potentials were seen from B6 only. However, the amplitude of the MRPs was smaller than in Fig 3. Epidurally recorded contralateral brain potentials (right side brain) were of significantly lower amplitude compared with subdurally recorded contralateral brain potentials shown in Fig 3

(a6, b6, a7, b7). These potentials were symmetrically localized contralateral to B6 and A7 and started about 700 ms prior to EMG onset. However, the NS′ and MP were poorly defined in these epidural recordings. The amplitude of these potentials at EMG onset was extremely small (-13.6 μV at b6).

Movement-related potentials associated with foot movements (Figs 6, 7, 8)

Movement-related potentials associated with left and right foot dorsiflexion were subdurally recorded from the left mesial surface in patients 1 and 2 (Figs 6, 7). Figure 8 highlights the waveforms at the critical electrodes (A7, B7, A8 and B8 in patient 1, and C4, D4, C5 and D5 in patient 2). With movements ipsilateral to the recording side in patient 1 (left side movements) (upper half of Fig. 6), a slow positive potential, BP, was seen at A7. A7 was located in the SMA and electrical stimulation elicited a positive motor response in the right upper and lower extremity. This BP was positive in polarity and started about 1400 ms before EMG onset. It was followed by a *positive* NS′ 300 ms before EMG onset with an amplitude of $+13.6$ μV. A positive MP started 100 ms before EMG onset, peaked 80 ms after EMG onset, and had an amplitude of $+73.6$ μV. The MP gradually reached the baseline level at 750 ms after EMG onset. In the primary motor foot area (A8, B8, A9, B9), neither a BP nor a NS′ was seen. However, positive

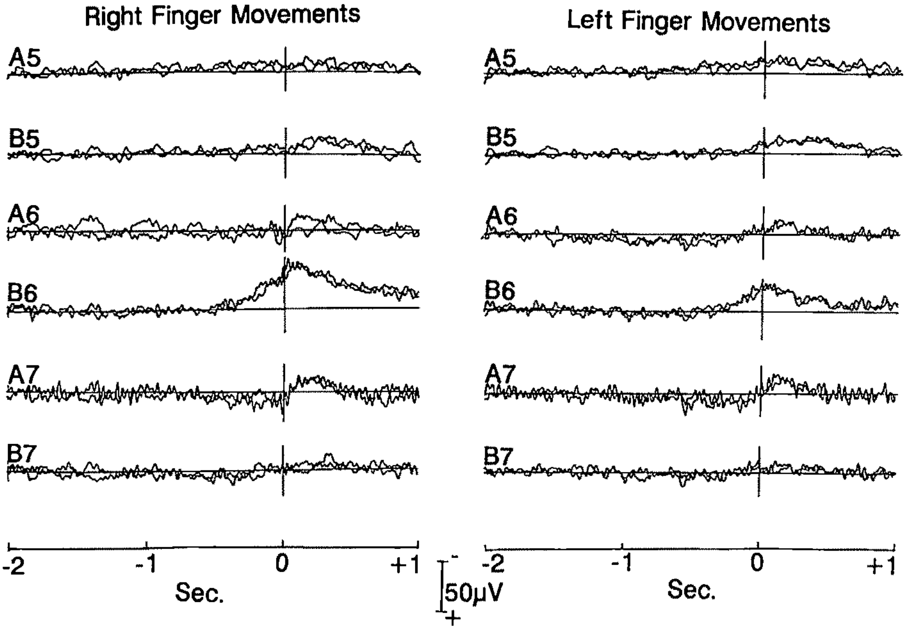

FIG. 5. Movement-related potential waveforms at the left SMA subdural electrodes (A5, B5, A6, B6, A7, B7) associated with right (Fig. 3) and left (Fig. 4) finger movements. It highlights the waveforms in the corresponding electrodes Negative BP, starting −600 ms, and NS′, starting −400 ms, were seen at B6 with both right (contralateral) and left (ipsilateral) finger movements. The BPs were of almost the same amplitude, and the NS′ with right finger movements was slightly larger than left movements Negative MP, starting −50 ms was present only with right finger movements

potentials starting just at EMG onset were seen at A8, B8, B7 and B9. The peak amplitude of these potentials was much smaller (+35 μV 100 ms after EMG onset at A8) than that at A7 (+73 μV at +100 ms).

With movements contralateral to the recording side in patient 1 (right side movements) (lower half of Fig. 6), a positive BP was seen in the SMA at the same electrode (A7) where a positive BP, NS′ and MP were seen with ipsilateral foot movements. The BP started about 1350 ms before EMG onset and it was followed by NS′ 300 ms before EMG onset with an amplitude of +19.1 μV. The onset of the MP was obscured by artefacts just coinciding with EMG onset. However, a clearly identifiable MP was seen just after EMG onset. This potential reached the baseline level slowly in the following 1 s. The time course of the MRP at A7 was almost identical for right and left foot movement. The amplitudes of BP and NS′ were, however, slightly larger with contralateral movements than with ipsilateral movements. With contralateral foot movements another positive BP was seen at the primary motor foot area (A8), starting about 1500 ms before EMG onset. It was followed by NS′ starting 360 ms before EMG onset with an amplitude of +21.8 μV. The onset of the MP was obscured by artefacts just at EMG onset, but an easily identifiable MP was seen afterwards. The time course of the MRPs and its amplitudes were almost identical at A7 and A8. A relatively short duration transient negativity occurred immediately after the artefact at EMG onset. This transient negativity was seen only at A8 and A9 and was superimposed on the ongoing

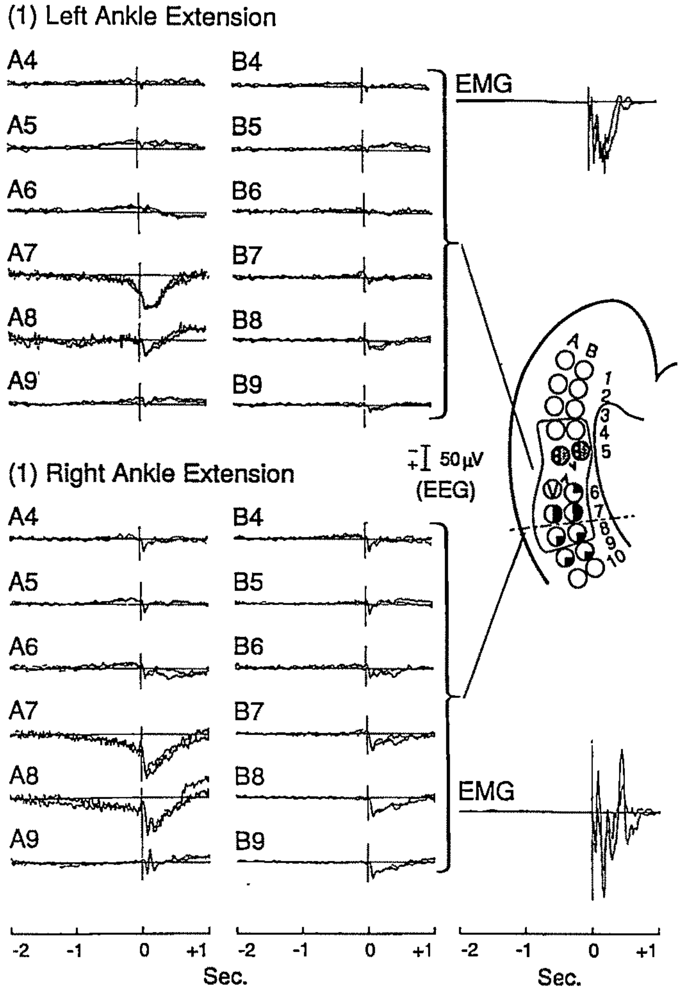

FIG. 6. Movement-related potentials associated with voluntary, self-paced foot dorsiflexions of the left (*upper half*) and the right (*lower half*) side recorded from the left mesial brain surface subdurally in patient 1. *Positive* BPs, NS' and MPs were of approximately equal amplitude at both SMAs (A7) and in the contralateral primary motor foot area (A8) A negative transient at +145 ms was seen only at the contralateral primary motor foot area (A8 and A9 in the lower half)

positive MP. It started 90 ms after EMG onset and peaked at 145 ms. This activity was absent in the SMA (A7), but was present only in the primary motor foot area (A8, A9). The MP which started after EMG onset was seen also at B7, B8, A9 and B9 but had lower amplitude. The MP with contralateral movements was more widely distributed (B7, B8, A9, B9) than with ipsilateral movements (A8, B8).

In patient 2, MRPs were recorded subdurally from the left mesial brain surface with ipsilateral and contralateral foot movements (Fig. 7). With movements ipsilateral to the recording side (left side movements), a positive BP was seen at D4, D3 and C3. These electrodes were placed in the SMA and electrical stimulation elicited positive

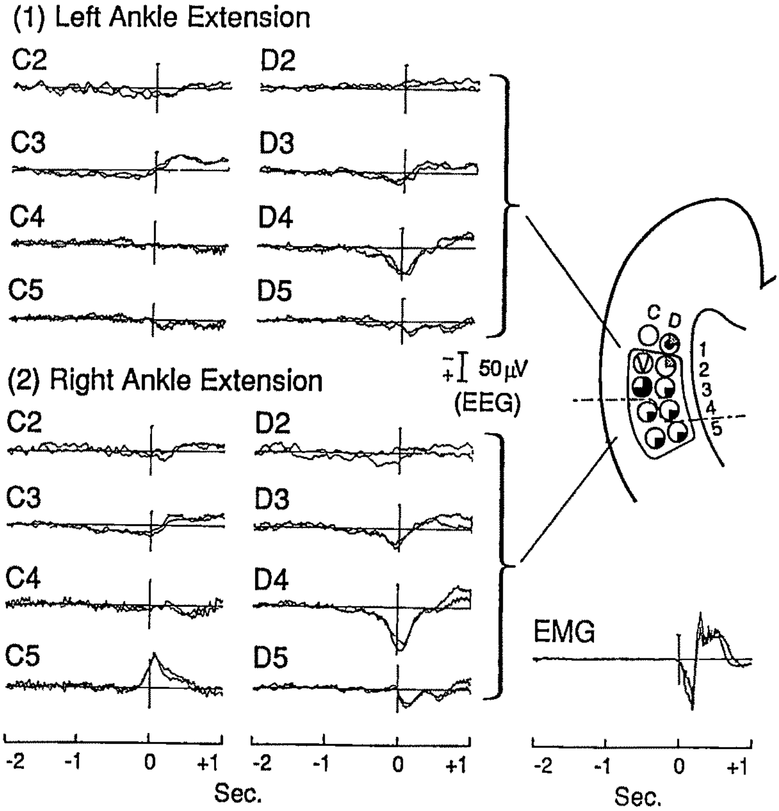

FIG. 7 Movement-related potentials associated with voluntary, self-paced foot dorsiflexions of the left (*upper half*) and the right (*lower half*) side recorded from the left mesial brain surface subdurally in patient 2. Negative MPs were seen only in the contralateral primary motor foot area (C5). In addition, *positive* BPs, NS′ and MPs of approximately equal amplitude were seen at both SMAs (D4)

motor response in the right and left lower extremities. At D4, the BP started about 700 ms before EMG onset and was followed by a positive NS′ 300 ms before EMG onset (+10.9 μV at D4). A positive MP started 100 ms before EMG onset and peaked about 100 ms after EMG onset with an amplitude of +47.7 μV at D4. Neither the BP nor the NS′ was seen at the other electrodes in the SMA or in any of the three electrodes in the primary motor foot area. However, an MP which started after EMG onset was seen at the primary motor foot area (C5, D5). This MP had a smaller amplitude (+21.8 μV at D5 140 ms after EMG onset). With movements contralateral to the recording side (right side movements) in patient 2, D4, D3 and C3 showed MRPs which had a time course almost identical to those with ipsilateral movements. The amplitude of the BPs was also very similar. However, the amplitudes of the NS′ and MP were significantly larger with contralateral movements than with ipsilateral movements. At D4, a positive BP started about 1300 ms before EMG onset, and a positive NS′ occurred at approximately 300 ms before EMG onset (+13.6 μV at D4). A positive MP started 120 ms before EMG onset and peaked 60 ms after EMG onset with an amplitude of

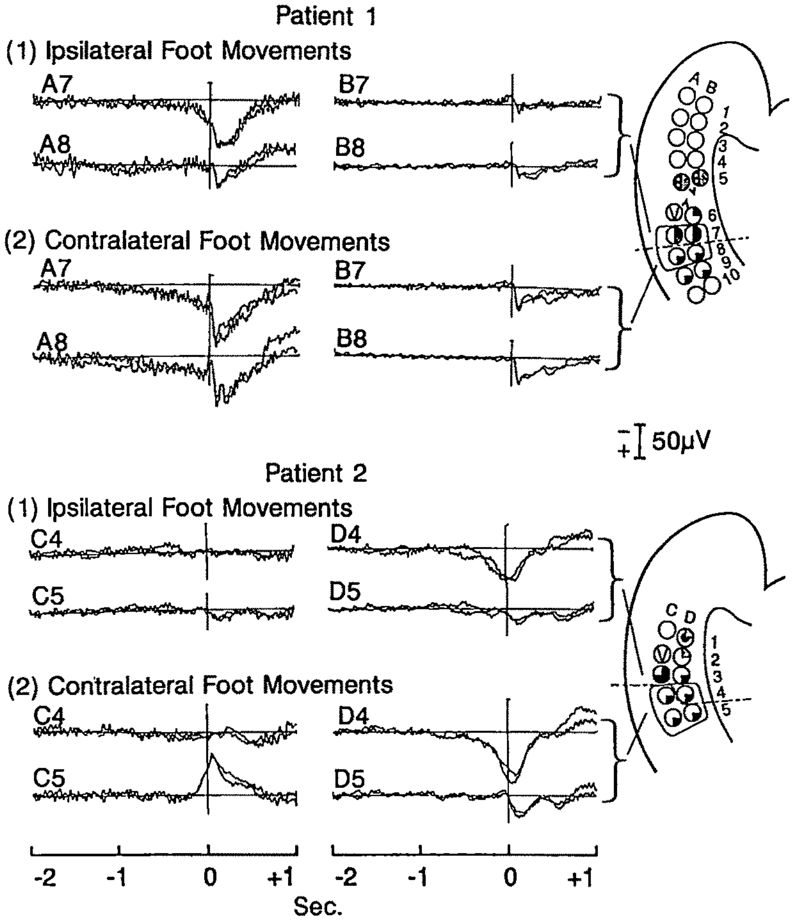

FIG 8. Movement-related potential waveforms at the left mesial brain in patient 1 (Fig. 6) and patient 2 (Fig. 7) associated with voluntary, self-paced left (ipsilateral) and right (contralateral) foot dorsiflexions. It highlights the waveforms at the critical subdural electrodes over the left SMA foot (A7 and B7 in patient 1, and D4 in patient 2) and primary motor foot (A8 and B8 in patient 1, and C4, C5 and D5 in patient 2) areas

+75 μV at D4. In addition, in contrast to ipsilateral movements, contralateral movements elicited a slow negative potential preceding EMG onset at the primary motor foot area (C5). The BP of the MRPs at C5 was poorly defined, but a relatively high amplitude NS′ started 300 ms before and an MP started 140 ms before EMG onset. The MP peaked 60 ms after EMG onset at exactly the same time as the MP at D4. The amplitude of the MP was −61.3 μV.

Movement-related potentials associated with tongue protrusions (Figs 9, 10)

Movement-related potentials associated with tongue protrusions were subdurally recorded from the left mesial surface in patients 1 and 2 (Figs 9, 10). In patient 1 (Fig. 9), a negative BP was widely distributed in the SMA over B4, A5, B5, A6, B6, A7 and B7. The most clearly defined peaks occurred at A6 and B5. These electrodes produced

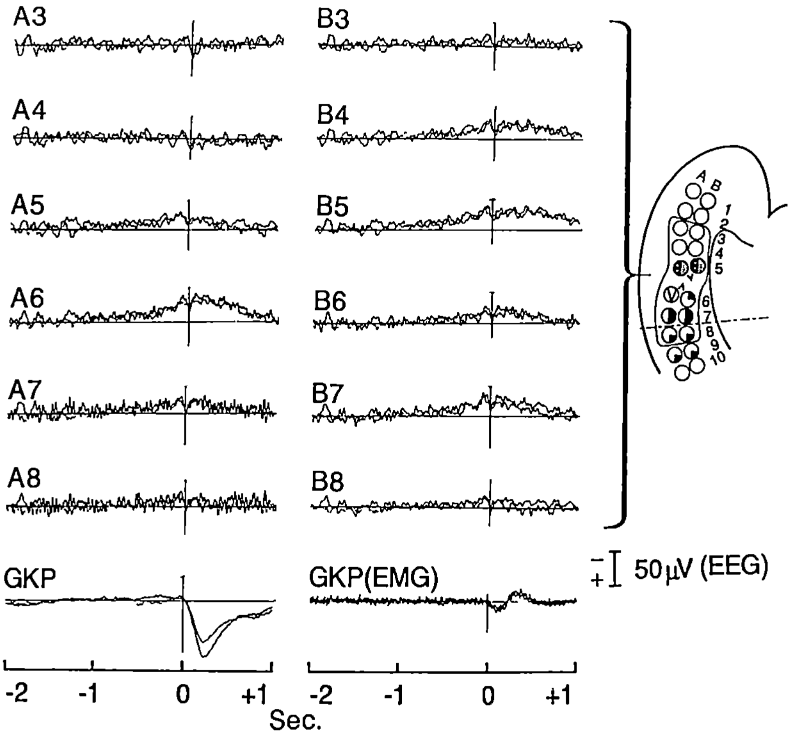

FIG. 9. Movement-related potentials associated with voluntary self-paced, tongue protrusions recorded from the left mesial brain surface subdurally in patient 1. Negative BPs, NS′ and MPs were seen anteriorly in the SMA (maximum at A6). GKP = glossokinetic potential.

vocalization and a positive motor response in the face, respectively, when stimulated. Very low amplitude potentials or no potentials at all were seen at B6, A7 and A8 where high amplitude MRPs were seen with finger (B6 in Fig. 3) and foot (A7 and A8 in Fig. 6) movements. At A6, the negative BP started 1400 ms before EMG onset and NS′ started 450 ms before EMG onset ($-10.9\ \mu V$ at A6). The slope became steeper at approximately 130 ms before EMG onset. This could correspond to the onset of the MP, but it was less well developed than with finger or foot movement (B6 in Fig. 3, A7 in Fig. 6). Then the MP consisted of a plateau ($-35.4\ \mu V$) until 500 ms after EMG onset. It then decayed very gradually.

In patient 2 (Fig. 10), a low amplitude positive BP was seen at C2 corresponding to the SMA which produced vocalization when stimulated. The BP started 1800 ms before EMG onset. However, no NS′ followed it and a negative MP started just at EMG onset. At D2 and C3, a clear BP did not occur but a *negative* NS′ started 400 ms before EMG onset and was followed by a negative MP without a clear transition point. The MP increased further in amplitude after EMG onset, peaked about 400 ms after EMG onset ($-54.5\ \mu V$ at D2) and then decayed gradually. These potentials were localized in the SMA at electrodes which, on stimulation, produced a positive motor response in the face (D2) and vocalization (C2). No potentials were seen at electrodes D4 and C5 which recorded the highest amplitude potentials with foot movements (Fig. 7). The

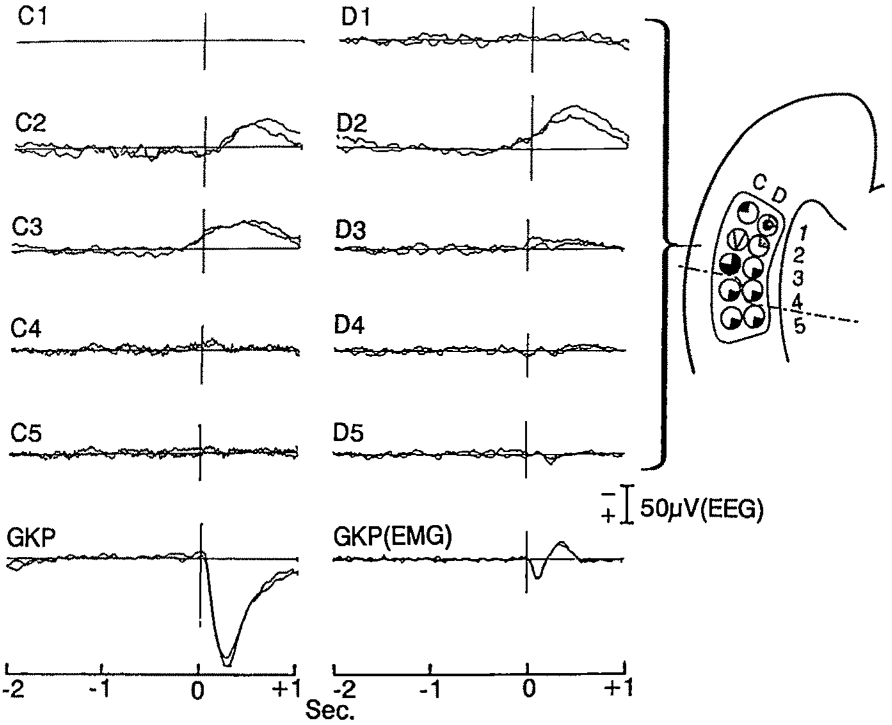

FIG. 10 Movement-related potentials associated with voluntary self-paced, tongue protrusions recorded from the left mesial brain surface subdurally in patient 2. A small positive BP at C2, and a clear *negative* NS′ at D2 and C3 was seen

average amplitude of NS′ with tongue movements 100 ms before EMG onset across the two patients was −23 μV, which was smaller than that with finger (−25.9 μV) and foot (+42.2 μV) movements, respectively.

Glossokinetic potential recording (employing EEG filter settings) did not record any potentials prior to the EMG onsets (tongue protrusions).

Movement-related potentials associated with vocalizations (Fig. 11)

Movement-related potentials associated with vocalizations were recorded in patient 2 (Fig. 11). A clear positive BP was noticed only at C2. It began 1500 ms before EMG onset and slowly increased in amplitude until 150 ms after EMG onset (+24.5 μV at EMG onset). It then decayed gradually, reaching the baseline level 500 ms after EMG onset. Electrode C2 was located in the SMA and electrical stimulation actually elicited rhythmically interrupted vocalization. In addition, a negative MP was seen at D2 and C3. It began at EMG onset and peaked 400 ms after EMG onset (−24.5 μV at D2). At D2 it continued for at least 1000 ms after EMG onset. The distribution and temporal evolution of the MRPs at C2, D2 and C3 was almost identical to one observed with tongue protrusion (Fig. 10). However, the amplitude of the preceding positive potential at C2 was larger with vocalization than that with tongue protrusion (+24.5 μV versus +13.6 μV, respectively, at EMG onset). No potentials were seen at D4 and C5 where the MRPs associated with foot movements had their highest amplitude (Fig. 7).

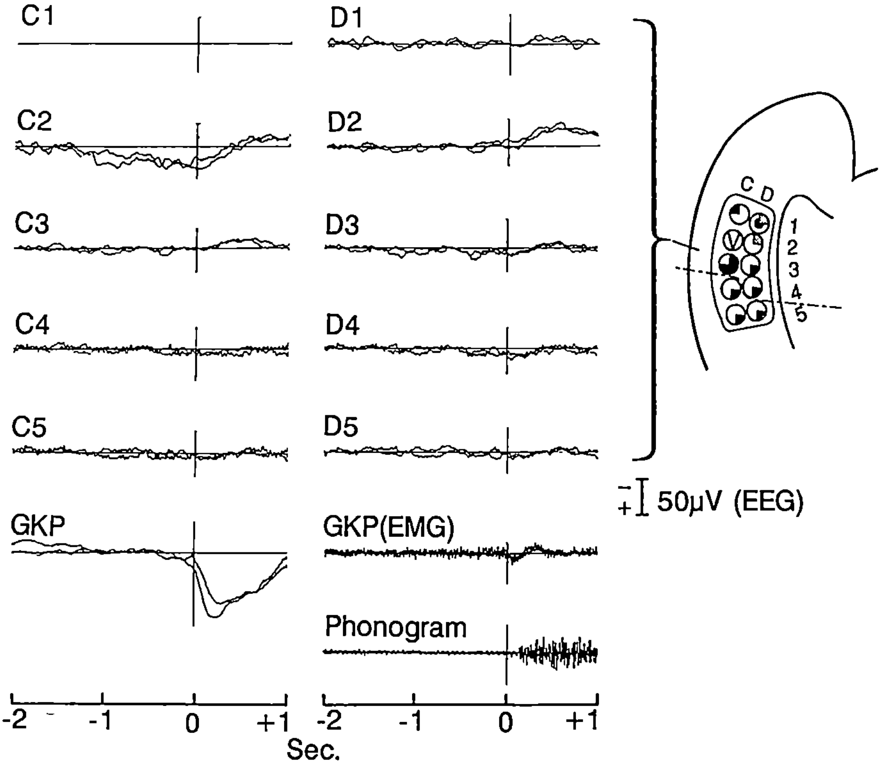

FIG 11. Movement-related potentials associated with voluntary self-paced, vocalization of the word 'pine' recorded from the left mesial brain surface subdurally in patient 2 Clearly defined positive BP was seen at C2 only.

Glossokinetic potential (recorded with EEG filter settings) and phonogram were also monitored simultaneously. They showed no potentials preceding EMG onset. The phonogram always occurred about 100–200 ms after EMG onset.

DISCUSSION

Movement-related potentials associated with foot movements (Figs 8, 13)

We reported here on recordings of MRPs associated with ipsilateral and contralateral foot movements from the primary motor foot area and from the SMA in two patients.

With movements contralateral to the recording side, clearly defined negative or positive BPs, NS′ and MPs were seen at the primary motor area in both patients. The temporal course of the MRPs elicited by foot movements in the primary motor foot area was consistent with that of MRPs elicited by finger movements and recorded from the primary sensorimotor cortex on the lateral convexity with subdural electrodes (Neshige *et al.*, 1988). Neshige *et al.* (1988) recorded slow negative potentials associated with finger movements at the primary sensorimotor finger area in six patients, and positive potentials anterior to the primary motor finger area in one patient. In our recordings these potentials were positive in one patient, and negative in the other patient. Bereitschaftspotentials

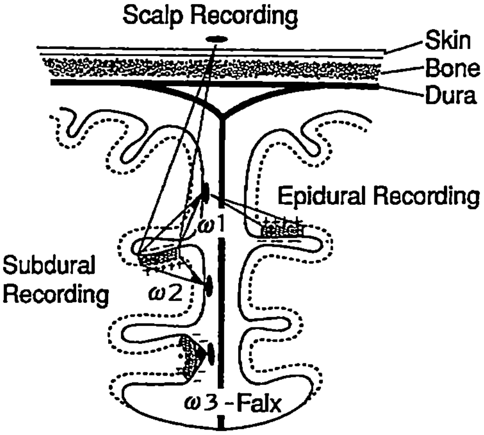

FIG. 12. Schematic representation of dipoles demonstrating the generators of MRPs in the mesial brain surface. Dipoles with a cortically superficial negative pole could be oriented in different directions depending on whether they are generated in the crown of the gyrus, or in the superior or inferior bank of a sulcus The diagram also shows a pair of subdural electrodes which could pick up the negative (ω1) or positive (ω2) pole of the dipole With a dipole located at the crown of the gyrus, only the negative pole would be picked up with subdural electrodes (ω3). The scalp electrode would only record summated potentials arising from several generators with solid angles which may be of opposite polarity. Scalp-recording potentials are also significantly attenuated by different volume conductors of relatively high resistance like bone, dura and skin.

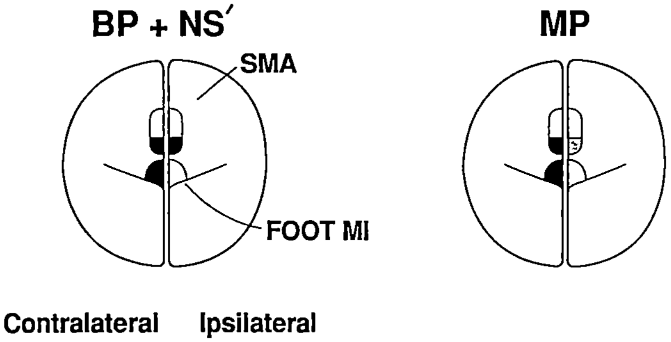

FIG. 13. Schematic representation of the cortical generators of BPs, NS′ and MPs for foot movements viewed from the top The degree of darkness of the shading in the SMA and MI is approximately proportional to the amplitude of the corresponding MRPs. MI = primary motor area.

recorded from scalp electrodes were usually of negative polarity, and positive BPs have been reported only on special occasions (Simpson and Khuraibet, 1987). Intracranial recordings with subdural as well as depth electrodes in experimental animals showed that the BPs or NS′ had a dipolar distribution with a negative pole at the surface and a positive pole in the deep grey matter. These findings were interpreted as an expression of excitatory postsynaptic potential currents generated in the superficial parts of apical dendrites of cortical pyramidal neurons (Arrezo and Vaughan, 1975, 1980; Hashimoto *et al.*, 1979; Sasaki *et al.*, 1979, 1981). Assuming that the BPs observed in these patients are generated by a similar mechanism, the positive potentials recorded here could be explained by the orientation of the dipole (Fig. 12). On the mesial surface, a dipole parallel to the interhemispheric fissure could be generated by infoldings of the cortex as shown in Fig. 12. Depending on the relative position of the electrode with respect to the dipole, the recordings would be of positive or negative polarity ($\omega 1$ and $\omega 2$ in Fig. 12).

With movements ipsilateral to the recording side, no potentials preceding EMG onset at the primary motor area were seen in either patient. Slow potentials were seen only after EMG onset with ipsilateral and contralateral foot movements. These results differ from the results of others who reported MRPs associated with ipsilateral and contralateral finger movements (Shibasaki *et al.*, 1980; Neshige *et al.*, 1988). In MRPs associated with finger movements recorded from subdural as well as scalp electrodes, a BP was recorded from both primary sensorimotor areas, whereas the NS′ and MP occurred mainly from the contralateral side. One possible explanation is that MRPs for foot and finger movements have a different generating process.

In this study we showed that high amplitude MRPs can be generated in the SMA. Bereitschaftspotentials and NS′ showed almost identical amplitudes for contralateral as well as ipsilateral movements. Motor potentials were also present in the SMA with ipsilateral and contralateral movements. However, MPs associated with ipsilateral movements were of smaller amplitude than those associated with contralateral movements. These results indicate that MRPs associated with foot movements originate from three generators, namely the contralateral primary motor area and both SMAs (Fig. 13). The BP and NS′ are of similar amplitude in these three generators. The MP, however, is generated mainly by the contralateral primary motor area and the contralateral SMA with a lesser degree of participation of the ipsilateral SMA. The ipsilateral primary motor area does not participate in the generation of any slow potentials preceding EMG onset.

Slow potentials just after EMG onset were seen with ipsilateral and contralateral foot movements. They were located close to electrodes where maximal BPs and NS′ were seen (Figs 6, 7). Those may represent one of MPs, either 'vicinity potentials' or 'somatosensory potentials' described by Neshige *et al.* (1988), which started at the time of, or immediately after, EMG onset. Those possibly reflect 'reafferent potentials' from muscle afferents described in scalp recordings, or actual participation of the adjacent brain tissue in ongoing voluntary movements (Kornhuber and Deecke, 1965).

Movement-related potentials associated with foot movements were studied previously by many investigators (Shibasaki *et al.*, 1981; Brunia and Bosch, 1984; Brunia *et al.*, 1985; Boschert and Deecke, 1986). They argued that the NS′ associated with foot movements was larger over the hemisphere ipsilateral to the movement, while the NS′ associated with finger movements was larger over the hemisphere contralateral to the movement. This 'paradoxical lateralization' of the NS′ associated with foot movements was explained by assuming that it was generated by a dipole located perpendicular to the mesial surface (Brunia and Bosch, 1984). This is the same explanation for the 'paradoxical lateralization' of somatosensory evoked potentials to posterior tibial nerve stimulation (Cruse *et al.*, 1982). From scalp recordings, however, we cannot expect to differentiate the MRPs generated in the primary motor area and in the SMA because of the immediate proximity of the two zones. Because of this proximity we would expect scalp recordings to reflect a complicated summation of several dipoles (Fig. 12). Invasive recordings, on the other hand, should be helpful in differentiating potentials recorded from the primary foot motor area and the SMA. From the results presented here, we concluded that the dipoles reflecting the MRPs associated with foot movements can be oriented in several different directions depending on whether they are generated in the crown, inside the sulcus of gyrus or somewhere in between (Fig. 12). The variable distribution and polarity of the MRPs recorded in this study also suggest that the cortical location of the different functional areas mentioned above might vary between individuals. These observations suggest that, when recording MRPs associated with foot movements from scalp electrodes, different generators consisting of dipoles of various orientations and strength are summated. The result is an unpredictable summation and cancellation of MRPs (Fig. 12). This may explain the controversy reported by previous investigators who studied the scalp topographical features of MRPs associated with foot movements (Shibasaki *et al.*, 1981; Brunia *et al.*, 1984, 1985).

Premotion positivity (PMP) which has been previously described in scalp recordings associated with finger movements (Deecke *et al.*, 1969; Shibasaki *et al.*, 1980 as P-50; Tarkka and Hallett, 1990) was not evident in this study. According to Shibasaki *et al.* (1980), the P-50 associated with finger movement is a small asymmetric potential which predominates ipsilaterally. It was assumed that this potential was a positive 'inhibitory potential' generated in the ipsilateral cortex. Neshige *et al.* (1988), however, found no PMPs when recording MRPs from subdural electrodes associated with finger movement. They concluded that the PMP recorded from scalp electrodes was most probably a reflection of the onset of MP superimposed on a decaying NS′. In our study, a PMP (a transient potential prior to EMG onset of opposite polarity to the BP) was not seen from either the bilateral primary motor foot areas or the bilateral SMAs. It is of interest that Shibasaki *et al.* (1981) failed to record a PMP associated with foot movement in eight normal volunteers using scalp electrodes.

In patient 1, a sharp transient negativity with a peak latency of 145 ms after EMG onset was seen at the primary motor area with contralateral foot movements, but not at the SMA (Fig. 6). This potential was not observed in MRPs associated with ipsilateral foot movements. It most likely reflects an early reafferent potential. Lee *et al.* (1986) described reafferent potentials in the primary sensorimotor area 18–32 ms after a photometer triggering in the MRPs associated with contralateral finger movements by subdural electrode recordings. These potentials had temporal and spatial features common to somatosensory evoked potentials to median nerve stimulation. In our recording, those early potentials reported by Lee *et al.* (1986) were not seen; the earliest transient activity started 92 ms after EMG onset. It is possible that this difference is related to the different recording settings used in the two sets of experiments; Lee *et al.* (1986) selectively filtered out frequencies of less than 1 Hz, and used a much shorter dwell time of 10–42 μs, thereby enhancing faster components compared with our recordings. In addition, the methods for obtaining the trigger pulse were also different; photometer versus EMG onset.

Movement-related potentials associated with finger movements (Figs 3, 4, 5, 14)

In the SMA, clearly defined BPs and NS′ were identified with contralateral as well as ipsilateral finger movements. Contralateral finger movements, however, elicited higher

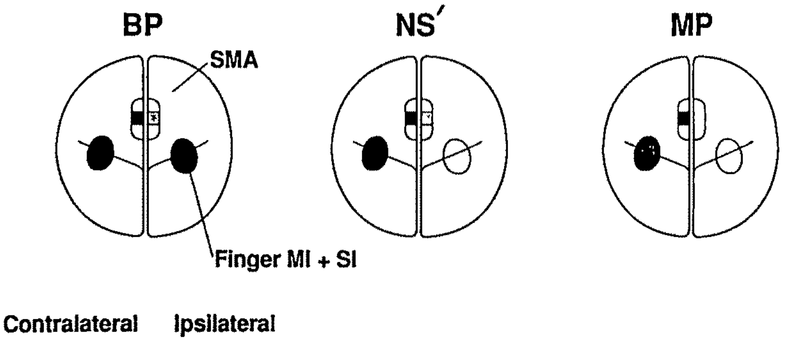

FIG 14 Schematic representation of the cortical generators of BPs, NS′ and MPs for finger movements viewed from the top. The diagram is based on the results obtained in this study and the previous results published by Neshige *et al* (1988). The degree of darkness of the shading in the SMA and MI is approximately proportional to the amplitude of the corresponding MRPs. SI = primary sensory area.

amplitude BPs and NS′ than ipsilateral finger movements. Motor potentials were also consistently seen with contralateral finger movements, but with ipsilateral finger movements MPs were either absent or of much smaller amplitude. These characteristics are almost identical to the features described for MRPs associated with foot movements in this paper. Movement-related potentials associated with finger movements were also extremely localized, occurring exactly at an electrode in the SMA where electrical stimulation had elicited contralateral upper extremity movements.

Movement-related potentials to contralateral finger movements recorded from epidural electrodes were widely distributed, but with an amplitude too small to identify individual components (right half of Fig. 4). No MRPs to ipsilateral finger movements could be identified from epidural recordings (right half of Fig. 3). These observations are in good agreement with previous reports from Neshige *et al.* (1988) who also recorded only low amplitude MRPs from the SMA with epidural electrodes. The low amplitude or absent MRPs in these recordings most probably resulted from the location of the epidural electrodes at a significantly greater distance from the generators than the subdural electrodes (Fig. 2).

Our observations complement a previous study by Neshige *et al.* (1988) regarding cortical generators of MRPs associated with finger movements. They reported that BPs arise from both primary sensorimotor finger areas, whereas the NS′ and MPs arise mainly from the contralateral primary finger area. Our results indicate that the contralateral SMA generates high amplitude BPs, NS′ and MPs which have a similar amplitude to those seen in the primary sensorimotor area. In addition, the ipsilateral SMA also generates BPs and NS′ of slightly lower amplitude, and essentially only very low amplitude MPs or none at all (Fig. 14).

Movement-related potentials associated with tongue protrusions and vocalizations (Figs 9, 10, 11)

With tongue protrusions, BPs, NS′ and MPs were also seen in the SMA. These potentials had a relatively wide distribution particularly when compared with the restricted distribution of BPs and NS′ seen with foot and finger movements. The maximal responses occurred at electrodes where electrical stimulation elicited positive motor response in the face or vocalization. The onset of the NS′ and MPs associated with tongue movements was not as clear as that with finger or foot movements. Movement-related potentials at the primary motor tongue area were not recorded simultaneously in the patients presented here. It is uncertain, therefore, whether MRPs recorded from the primary motor tongue area are as large as those seen in the SMA.

Bereitschaftspotentials associated with vocalizations were consistently of positive polarity and had a distribution similar to BPs associated with tongue protrusions (Figs 10, 11); the maximal response occurred at electrodes corresponding to the positive motor area for vocalizations. The existence of MRPs associated with vocalizations in scalp recordings has been reported by many investigators previously (Vaughan *et al.*, 1968; McAdam and Whitaker, 1971; Grözinger *et al.*, 1975, 1980; Deecke *et al.*, 1986). However, in recordings from scalp electrodes, artefacts preceding vocalizations such as GKP and the effects of respiration cannot be eliminated completely (Grözinger *et al.*, 1975, 1977, 1979; Szirtes and Vaughan, 1977; Brooker and Donald, 1980). The positive polarity of the BPs associated with vocalizations as well as with tongue protrusion could be an expression of the orientation of a dipole located parallel to the mesial surface as discussed previously in this paper (Fig. 12).

It has been argued before that the SMA participates in speech production. Grözinger *et al.* (1980) argued that BPs associated with speech production were of significantly larger amplitude at the vertex than at the lateral convexity. This difference of amplitude of BPs was more pronounced for MRPs associated with vocalization than those for other voluntary movements. Studies of regional cerebral blood flow (Orgogozo and Larsen, 1979) also suggested almost exclusive activation of the SMA during speech production. However, speech production is always accompanied by tongue movements, and the data shown here revealed that MRPs associated with tongue movements and with speech production had similar patterns of temporal evolution and almost identical spatial distributions in the SMA. It is tempting to conclude, therefore, that MRPs associated with speech production are most likely associated with the accompanying tongue movements and are not directly related to language function.

Movement-related potentials in SMA (Figs 15, 16)

Previous investigators studying MRPs thought that the SMA was one of the main generators of the BPs and NS′. They speculated that the SMA works as a ‘supramotor’ area that initiates and regulates voluntary movements contributing to the generation of new motor programs and to the control of the execution of established movements (Boschert *et al.*, 1983; Goldberg, 1985; Regan, 1989; Porter, 1990). Experimental neurophysiological studies in animals showed that numerous SMA neurons were active in association with the execution of voluntary limb movements (Neafsey *et al.*, 1978; Brinkman and Porter, 1979; Smith, 1979; Tanji and Kurata, 1979; Wise and Tanji, 1981), but that the intensity of the movement-associated neuronal activity in the SMA was distinctly less than in the pre-central motor area (Brinkman and Porter, 1979; Tanji and Kurata, 1982). Kurata and Tanji (1985) and Tanji and Kurata (1985) showed that SMA neurons exhibited differential responses depending on whether the animal was prepared to press the key in response to an auditory signal or remained motionless in response to a somatosensory signal. In both instances the precentral motor area neurons showed similar responses. This suggests that the SMA is more active than the primary

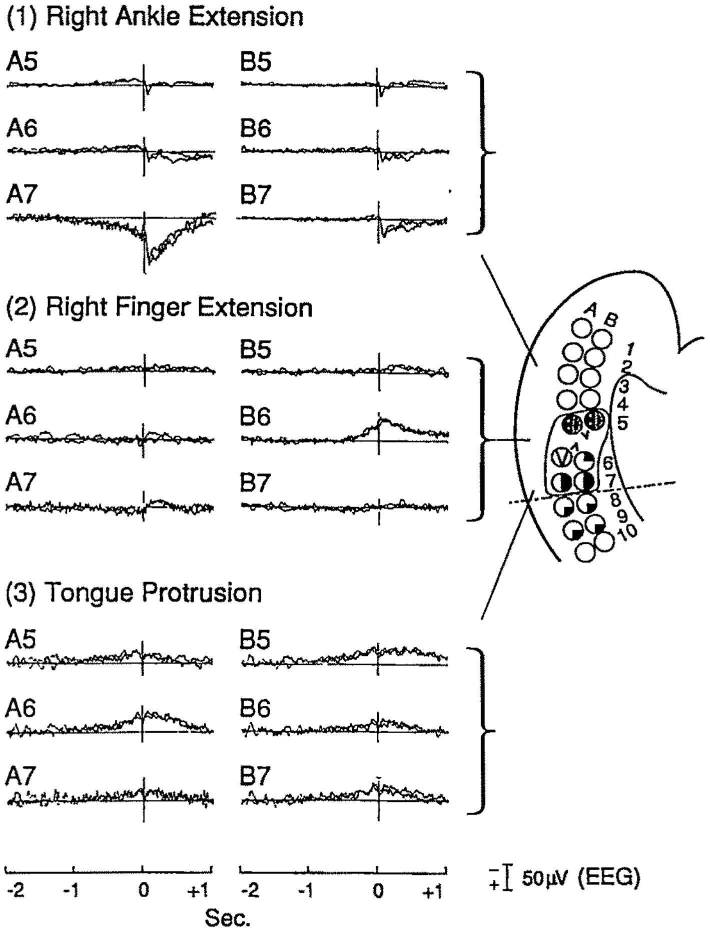

FIG. 15 Movement-related potentials associated with voluntary movements of finger, foot and tongue recorded from the SMA in patient 1. The figure illustrates the somatotopic organization of the MRPs in the SMA

motor area in relation to complex motor performances as compared with the execution of simple movements. Neafsey *et al.* (1978) also showed that the neuronal activity differed between the SMA and the precentral motor area prior to initiation of different voluntary movements in cats. Cerebral blood flow studies in human cortex also suggested that the SMA is significantly more activated in programming complicated motor performances rather than in executing simple movements (Orgogozo and Larsen, 1979; Roland *et al.*, 1980).

The early components of the BP associated with voluntary eye, arm, hand or foot movements recorded from scalp electrodes are always symmetrically distributed with the maximum amplitude at the vertex (Kornhuber and Deecke, 1965; Vaughan *et al.*, 1968; Shibasaki *et al.*, 1980; Boschert and Deecke, 1986). This distribution was interpreted as indicating that the SMA is the generator of the early part of the BP (Deecke *et al.*, 1985*b*; Boschert and Deecke, 1986). Movement-related potentials studied in

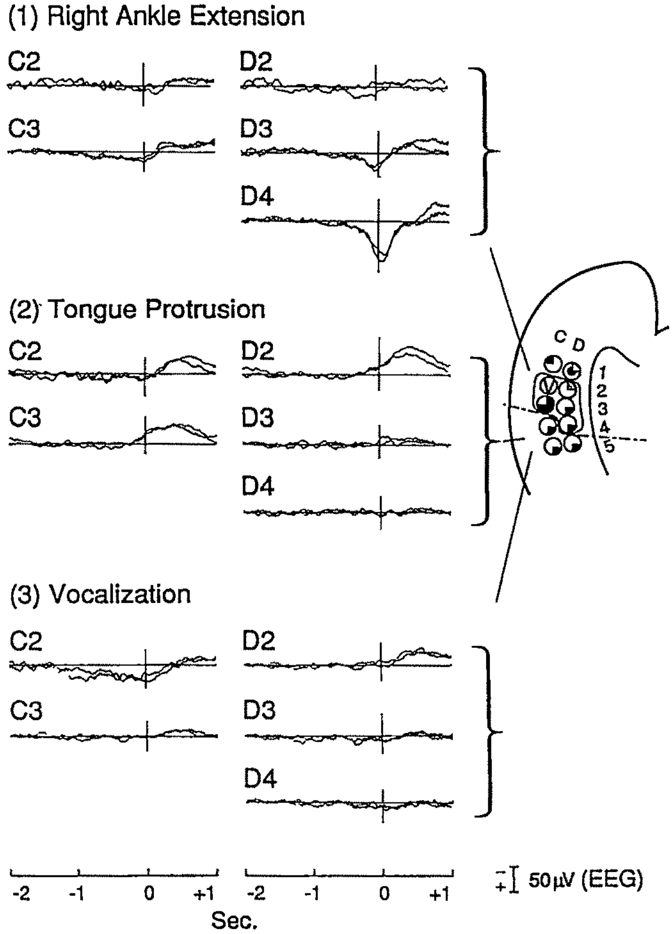

FIG. 16. Movement-related potentials associated with foot and tongue movement and vocalization recorded from the SMA in patient 2. The maximal response of the MRPs for tongue movement and vocalization occurred at the same electrodes (C2, D2, C3), whereas the maximal response of the MRPs associated with foot movement was seen at a different electrode (D4). This figure also illustrates the somatotopic organization of the SMA

parkinsonian patients also suggested that there were independent generators for the BP in the SMA and in the primary motor area (Deecke and Kornhuber, 1978; Dick *et al.*, 1989). Extracranial magnetoencephalographic studies also suggested two generators for complicated movements: one for the earlier portion of the BP in the SMA and the other for the later portion of the MP in the primary motor area (Deecke *et al.*, 1985*a*). A recent study of MRPs recorded from subdural and epidural electrodes in human cortex (Neshige *et al.*, 1988) suggested, however, that scalp-recorded BPs are the result of a summation of BPs arising mainly from bilateral primary sensorimotor areas. This could explain the symmetric distribution of the early phase of the BPs without significant participation of the SMA. It is important to remember here, however, that their recording of the BPs from the SMA was obtained with epidural electrodes which tend to be of significantly lower amplitude than the corresponding subdural recording.

In this study we found that the SMA generates MRPs (BPs, NS′, MPs) which are of similar amplitude to the MRPs generated in the primary motor area. With finger and foot movements, both SMAs generated BPs of approximately equal amplitude, whereas NS′ and MPs were of higher amplitude in the contralateral SMA than in the ipsilateral SMA. This study also showed that the MRPs associated with voluntary movements of finger, foot and tongue, and with vocalization had a somatotopic distribution in the SMA which corresponded approximately to the somatotopic organization demonstrated by electrical stimulation. Movement-related potentials associated with finger, foot and tongue movements were arranged in an anterior-posterior row within the SMA. This distribution of the MRPs corresponds to the somatotopic organization of the SMA described in monkeys (Woolsey *et al.*, 1952) and more recent human studies (Talairach and Bancaud, 1966; Fried *et al.*, 1991).

Deecke (1990) hypothesized that the SMA generates the early phase of the BP, which would be related to motivational, intentional or timing properties particularly of complicated tasks or during the learning process. The primary motor area would generate the late phase of the BP and NS′, which would be related to the actual preparation of the final pyramidal tract volley. In our study, the BP and NS′ associated with foot movements were of similar amplitude and appeared at approximately the same time in the SMA and in the primary foot area. This was also the case for relatively complex movements like vocalization where a complicated sequential participation of muscles of the larynx, pharynx and mouth was required. It did not differ significantly from simpler movements like tongue protrusions. These results, therefore, do not suggest the hypothesis of a ‘supramotor’ function for the SMA. Both SMAs seem to play a significant role in the organization of voluntary movements, generating MRPs which have a similar pattern of evolution over time and somatotopic organization to that in the primary motor area. These findings suggest primarily a ‘supplementary’ function for the SMA. However, additional studies will have to be performed to exclude the possibility that the SMA has a ‘supramotor’ function for more complicated sequential finger or foot movements, particularly for movements in which a learning process is involved.

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EFFECTS OF FOCAL TRANSCRANIAL MAGNETIC STIMULATION ON SIMPLE REACTION TIME TO ACOUSTIC, VISUAL AND SOMATOSENSORY STIMULI

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SUMMARY

In a simple reaction time (RT) paradigm, magnetic stimulation of different intensities was delivered over different scalp positions and at variable delays before (negative) or after (positive) the go-signal. Magnetic stimulation shortened RT to different go-signals (auditory, visual and somatosensory stimuli) by approximately 30 ms when delivered over the motor cortex contralateral to the responding arm at intensities below motor threshold. This effect was maximal at a delay of approximately +10 ms. A similar effect was found with suprathreshold stimulation to the ipsilateral motor cortex. Magnetic stimulation over other scalp areas did not affect RT regardless of the delay. No differences were found between the effects on elbow flexion and thumb abduction. The shortening of RT was not associated with changes in the timing development of premovement excitability increase in the motor cortex. We conclude that magnetic stimulation shortens RT by inducing an earlier initiation of this excitability increase.

INTRODUCTION

When simple reaction time (RT) to focal transcranial stimulation was compared with RT to acoustic, visual and somatosensory stimuli (Pascual-Leone *et al.*, 1992), RT was longest to a magnetic or electrical stimulus delivered over the contralateral motor cortex at an intensity high enough to induce motor evoked potentials (MEPs) in muscles involved in the response (suprathreshold intensity). Conversely, RT was shortest to subthreshold transcranial stimulation over the same scalp position. This report describes the effects of transcranial magnetic stimulation (TMS), to which the subjects are not supposed to respond, on RT to acoustic, visual or somatosensory stimuli (go-signal).

In RT paradigms, transcranial stimulation properly delivered in time and space can modify the onset latencies of the responses. Reaction time may be prolonged or shortened depending on the intensity of the stimulus. Suprathreshold transcranial stimuli can delay RT to an auditory go-signal if delivered over the motor cortex close to the expected response time (Day *et al.*, 1989). Transcranial weak direct currents applied over the sensorimotor cortex can shorten RT to go-signals of different modalities (Elbert *et al.*, 1981; Jaeger *et al.*, 1987).

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Reaction time can be divided into a first period during which the motor cortex is less excitable, and a second period during which it becomes increasingly excitable, leading to movement onset (Starr *et al.*, 1988). Hallett *et al.* (1991) have shown that the study of the relative duration of these two periods can provide some understanding of RT abnormalities in patients with Parkinson's disease. Similarly, the effects of transcranial stimulation on RT to different go-signals may be clarified by comparing the duration of these two RT periods in trials with and without transcranial stimulation.

METHODS

Reaction time experiments

We studied five naive, right-handed normal volunteers (three men and two women), aged 26–42 yrs An auditory warning signal, used to alert the subject, was followed at random intervals (foreperiod, 1–5 s) by an auditory, visual or somatosensory go-signal. In response to the go-signal, the subject flexed the right elbow or abducted the right thumb as rapidly as possible. When the response was elbow flexion, the subjects were seated comfortably on a chair with the right arm slightly abducted at the shoulder and flexed 90° at the elbow so that the pronated forearm rested on a horizontal platform. When the response was thumb abduction, the subjects were seated with the right hand supinated and resting on a horizontal platform, the thumb adducted and the elbow flexed at 90°.

Each subject completed sets of trials using three different go-signals: a click, a flash and an electrical stimulus to the left index finger. The click was generated by a Grass auditory stimulator and delivered by a loudspeaker suspended 15–20 cm over the subject's head. The flash was generated by a Grass PS22 photic stimulator and delivered at an intensity of 100% of the stimulator's output by a lamp positioned at eye level 30 cm in front of the subject. The electrical stimulus was generated by a Grass electric stimulator and delivered by two surface electrodes taped 3 cm apart to the subject's left index finger. The intensity of the electric stimulus was kept at three times sensory threshold as determined by the method of limits (Gescheider, 1976).

Reaction time was measured from the go-signal to the onset of biceps or abductor pollicis brevis (APB) electromyographic (EMG) activity. The EMG was recorded with two surface electrodes taped over the muscle belly. The EMG signal was amplified and filtered (100–2000 Hz) by Grass amplifiers, digitized with a sampling rate of 5000 Hz per channel and rectified. The device delivering the go-signal was triggered with a 100 ms delay after EMG recording began; the total sweep time was −100 to 400 ms. All data were collected using an AST personal computer.

Transcranial magnetic stimulation was delivered with a Cadwell MES 10 magnetic stimulator equipped with an 8-shaped coil in which each component measured 4 5 cm in diameter. The coil was held flat on the scalp over the position at which TMS induced MEPs of maximal amplitude in the contralateral biceps or APB. (These positions were determined, with the patient at rest, by delivering TMS at an intensity of 100% of the stimulator's output over different scalp areas several times during the experiment.) The handle of the coil was held parallel to the sagittal axis of the subject's head, pointing occipitally. This technique allows relatively focal cortical stimulation (Cohen *et al.*, 1990); the characteristics of the electric field induced in the cortex are discussed elsewhere (Roth *et al.*, 1991). Subthreshold intensity is the highest intensity that did not evoke a MEP in the target muscle at rest (at a recording sensitivity of 50 μV/division) in five trials.

The experiments were performed in sets of 12 trials presented in random order. In each set, one-third of the trials were control trials (go-signal only), one-third were test trials (go-signal plus TMS) and one-third were catch trials (TMS only). In the test trials, TMS was delivered before (negative) or after (positive) the go-signal; delay ranged from −50 ms to +50 ms and was randomly varied in the different trials. The catch trials served to ensure that the subjects were responding to the go-signals and not TMS.

The study of each subject was completed in three different recording sessions of 11 sets each (132 trials) using a single go-signal in each session. This procedure was intended to avoid fatigue. The order of the recording sessions for the different go-signals was varied in different subjects. The first set of 12 trials in each recording session was considered a practice set and discarded from analysis. During the practice trials, the subjects were encouraged to perform as rapidly as possible, thus minimizing RT variability

For each subject, we measured RT in the control trials and calculated a mean and standard deviation of RT for each go-signal. In the test trials, we measured RT and calculated a mean and standard deviation of RT for each delay tested. Results across subjects were compared with one-way analysis of variance (ANOVA) repeated for the different go-signals. Comparison of RTs to the different go-signals in control and test trials was performed using one-way ANOVAs collapsing across subjects. Significance level, tested with Scheffé's test, was set at $P < 0.05$.

We also studied the effects of variable intensities of TMS delivered concurrently with the go-signal (delay = 0). The initial stimulus, delivered at subthreshold intensity, was randomly increased or decreased stepwise by 5% of the stimulator's output. Finally, to evaluate the topographic specificity of the effects of TMS on RT, we delivered TMS to F3/4, ipsilateral motor cortex, or P3/4 concurrently with a visual go-signal (delay = 0).

Motor cortex excitability experiments

The experimental design was the same as for the RT experiments involving thumb abduction, and the same subjects were studied. The study of APB allowed us to compare our results with those of Starr *et al.* (1988). In half of the trials, we used the same visual go-signal as in the RT experiments (control trials). In the other half, the go-signal was the same visual stimulus coupled with a subthreshold TMS delivered to the ideal position for evoking MEPs in the contralateral APB (test trials). In both control and test trials, a subthreshold TMS (probing stimulus, S) was delivered at variable times during the RT to assess the probability of evoking MEPs in the APB as a function of the proximity of voluntary EMG onset. The MEP amplitude was expressed as a percentage of the maximal M-response following peripheral electrical nerve stimulation. In the test trials, S was identical in intensity and localization to the TMS coupled with the visual stimulus as part of the go-signal.

Transcranial magnetic stimulation was delivered with a Cadwell magnetic stimulator capable of delivering single or twin pulses at intervals as short as 30 ms without changes in the amplitude of the pulse. Technical information about this stimulator is presented elsewhere (Pascual-Leone *et al.*, 1991, Appendix 1). The stimulation coil and its position on the scalp were the same as in the RT experiments.

In each subject, we recorded 120 trials (60 control and 60 test trials) in a single recording session. We compared RT in control trials with RT in test trials using two-way ANOVA (subject and trial type). To analyse the probability of S evoking an MEP, we aligned the trials at EMG onset (response) and expressed the probability as a function of the interval between S and EMG onset. We compared the probability curves in the control trials with those in the test trials to assess the effect of TMS in the go-signal (test trials) on the build-up of motor cortex excitability during RT.

RESULTS

Reaction time experiments

All subjects occasionally responded to isolated catch trials (Fig. 1). When this occurred, the entire set of 12 trials was discarded from further analysis because of the possibility that the subject was responding to the magnetic stimulus rather than to the go-signal in the test trials. Errors in catch trials occurred regardless of the go-signal modality, but were significantly more frequent when the go-signal was auditory (Fig. 1).

Reaction time with TMS (test trials) was shorter ($P < 0.001$) than RT without TMS (control trials) regardless of the go-signal modality. The difference between RT in control and test trials, considered to be the amount of RT shortening due to TMS, was approximately 30 ms regardless of the go-signal modality. The results for elbow flexion and for thumb abduction were similar (Table 1). The shortening of RT by TMS started at a delay of −30 ms, was maximal at +5 ms to +10 ms, and lasted up a delay of +30 ms. When the delay was +50 ms or longer, RT was prolonged rather than shortened. The relationship between RT shortening and delay was the same regardless of the

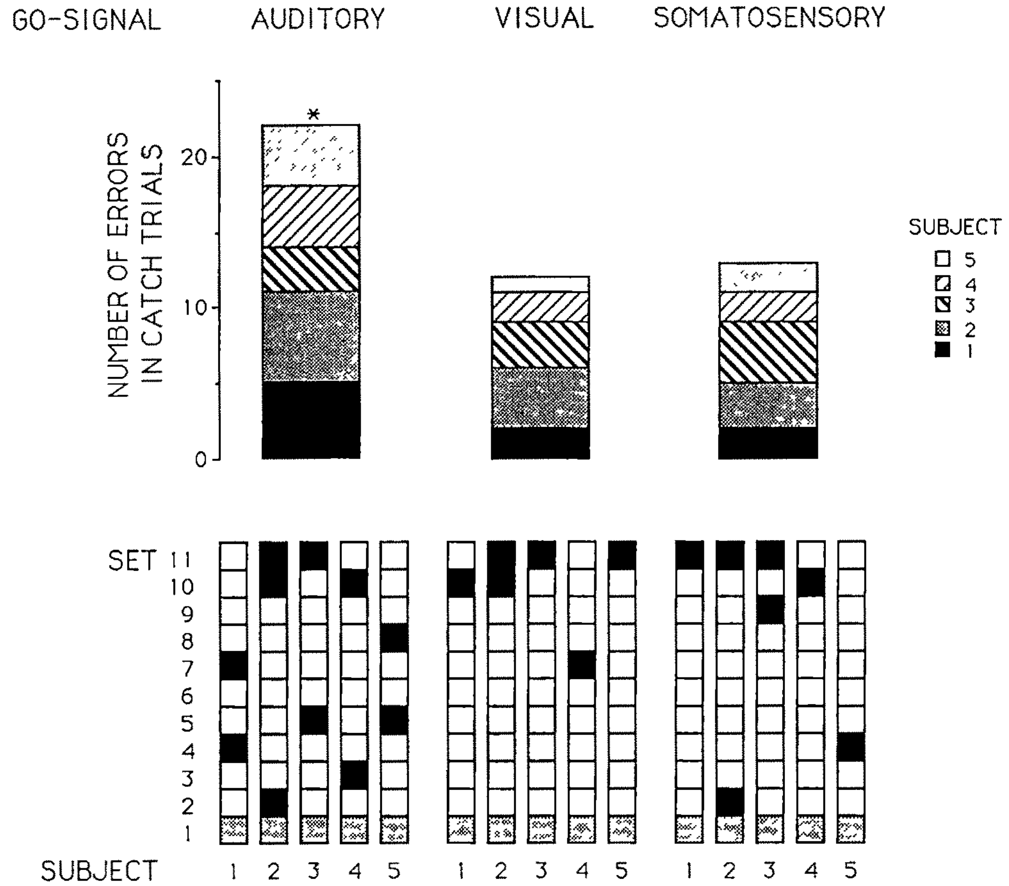

FIG 1. Sets of arm flexion RT experiments for each subject and go-signal modality (*bottom*) and stacked bar-histogram of the number of errors in catch trials according to the go-signal modality (*top*). The experiments were organized in 11 sets of 12 trials containing four control, four test and four catch trials. If the subject committed an error in a catch trial, the entire set was discarded (closed squares). The first set in each experiment was considered practice and was also discarded (stippled squares). Results are based on the remaining sets (open squares) Significantly more errors were committed by the subjects when an auditory go-signal was used (*$P < 0.05$). The results were essentially the same when the response was thumb abduction

TABLE 1. SHORTENING OF RT BY SUBTHRESHOLD TMS TO THE CONTRALATERAL MOTOR CORTEX

	Reaction time (ms)					
	Arm flexion			*Thumb abduction*		
Go-signals	*Control trials*	*Test trials*	*Difference*	*Control trials*	*Test trials*	*Difference*
Auditory	124.1 ± 11.1	91.6 ± 7.1*	32.5 ± 13.2	129.2 ± 13.5	94.6 ± 9.7*	34.6 ± 14.1
Visual	150.3 ± 10.2	121.7 ± 9.2*	28.6 ± 12.3	154.4 ± 11.8	123.6 ± 17.9*	30.8 ± 18.1
Somatosensory	137.4 ± 9.4	106.3 ± 9.6*	31.1 ± 10.2	139.9 ± 14.8	106.3 ± 13.6*	33.6 ± 15.2

Values are mean ± SD of the total number of trials in all five subjects. *$P < 0.001$ for comparison with control trials

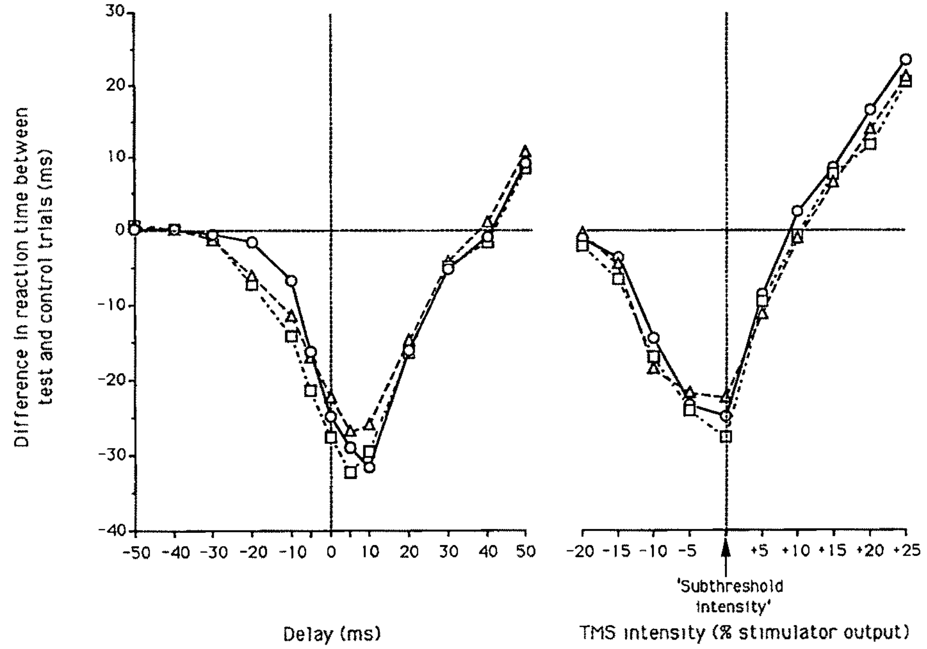

FIG. 2. Effect of TMS on arm flexion RT according to the interval between go-signal and TMS (delay, *left*) and according to TMS intensity (delay = 0, *right*). Symbols represent different go-signals: open circles = somatosensory, open triangles = visual, open squares = auditory. Maximal shortening of RT occurs at delays of +5 ms to +10 ms and subthreshold intensity. Subthreshold intensity refers to the intensity at which no biceps MEP was recorded (sensitivity 50 μV/division) in any of five trials with the subject at rest when TMS was delivered over the ideal scalp position for biceps MEP. Note that TMS at a delay of +50 ms and at suprathreshold intensities prolonged RT regardless of go-signal modality.

go-signal modality (Figs 2, 3). With concurrent delivery of the go-signal and TMS, the effect of TMS to shorten the RT decreased at intensities below subthreshold. At intensities above subthreshold, TMS prolonged the RT (Figs 2, 3).

There were no significant differences for any of these effects regardless of whether the tested movement was elbow flexion or thumb abduction. There was, however, a tendency for the effects of TMS to be more prominent in the APB (Table 1 and Fig. 4).

Reaction time to the auditory go-signal was shortest, followed by the somatosensory go-signal and then the visual go-signal (Table 1). These results were predicted by our previous study (Pascual-Leone *et al.*, 1992) and will not be further discussed here.

We found no significant differences between RT in trials without TMS and trials with TMS over F3, F4, P3 or P4. Transcranial magnetic stimulation over the ipsilateral motor area did not affect RT when delivered at subthreshold intensities; however, it shortened RT when delivered at suprathreshold intensities (Fig. 5).

Motor cortex excitability experiments

Reaction time in control trials was longer than in test trials (154.4 ± 19.7 ms versus 124.1 ± 13.2 ms) ($P < 0.001$). The probing stimulus S never elicited a MEP when

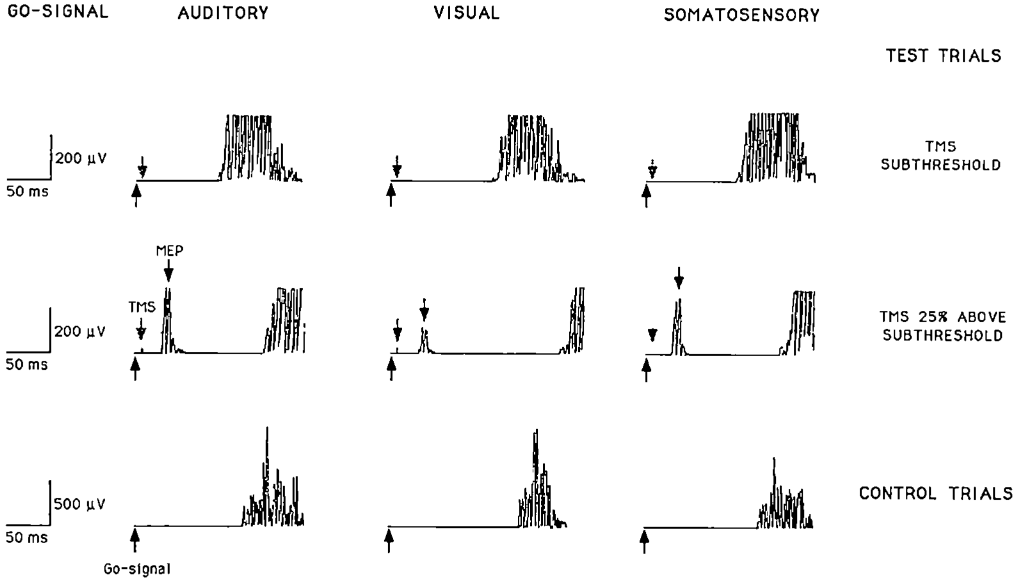

FIG. 3. Representative EMG recorded during RT for the different go-signals without TMS and with TMS at subthreshold and suprathreshold intensities (delay = 5 ms).

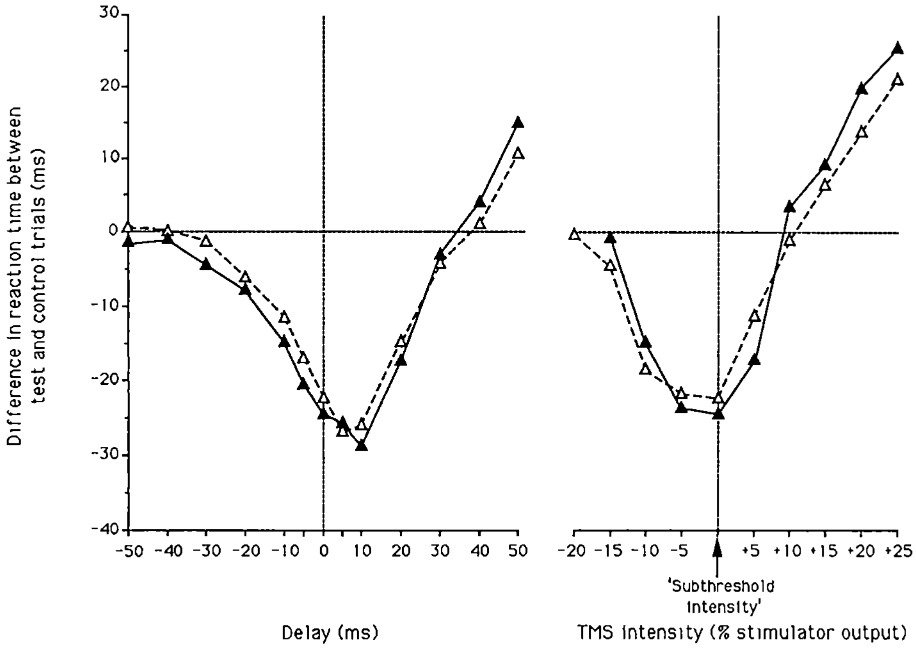

FIG. 4. Comparison of the effects of TMS on RT to a visual go-signal for arm flexion (open triangles) and thumb abduction (closed triangles) according to the interval between go-signal and TMS (delay, *left*) and according to TMS intensity (delay = 0, *right*). The effects on RTs to auditory and somatosensory go-signals were equally similar

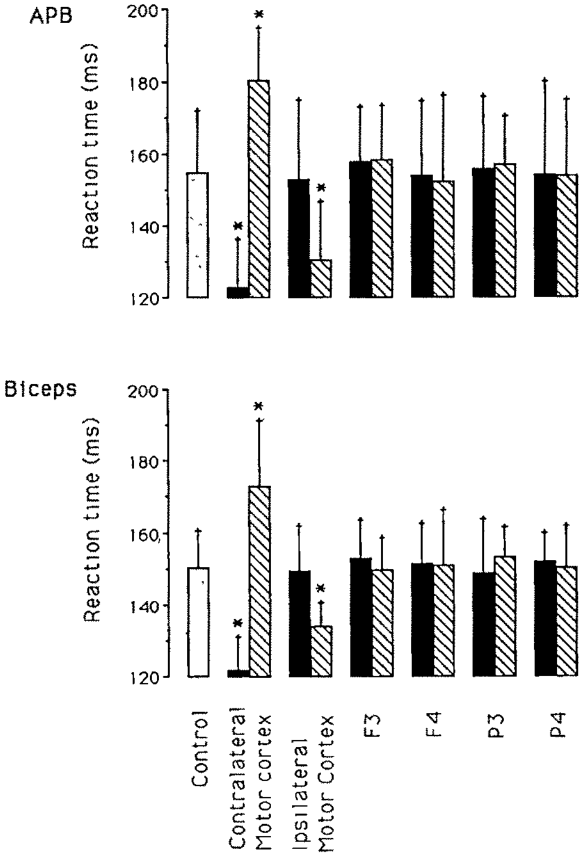

FIG 5 Effect of TMS to different scalp areas on RT (mean ± SD) to visual go-signal. Stippled bars represent RT in trials without TMS (control), closed bars represent trials with subthreshold TMS, hatched bars represent trials with TMS at 25% suprathreshold intensity Note the similarity of the results for elbow flexion (biceps) and thumb abduction (APB). *$P < 0.01$

the interval between S and EMG onset was ≥80 ms in either control or test trials (Fig. 6). With intervals of <80 ms, the probability of inducing a MEP gradually increased and reached 1.0 at intervals ≤30 ms in both control and test trials (Fig. 7).

The amplitude of the evoked MEPs increased rapidly with shorter S-EMG onset intervals in control and test trials (Figs 6, 8).

DISCUSSION

Our results expand the findings of Day *et al.* (1989) on the effects of TMS on RT. We have confirmed that prolongation of RT will result from stimuli of suprathreshold intensity and stimuli delivered close to voluntary EMG onset. In addition, we have found shortening of RT by stimuli of subthreshold intensity to the contralateral motor cortex

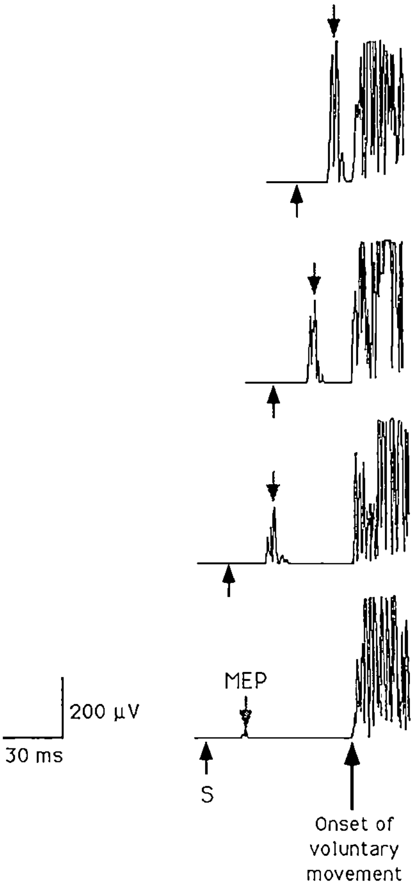

Fig. 6. Representative examples of test trials in motor cortex excitability experiments. Note the relationship between the amplitude of the MEP and the interval between probing stimulus S and EMG onset.

delivered early during the RT, and by suprathreshold stimuli to the ipsilateral motor cortex.

Theoretical model of response preparation and RT

The effects of TMS on RT can be explained using a model of response preparation. We divide the processes required for response preparation and execution into a stimulus evaluation system, a task-specific circuitry and a response channel. The stimulus evaluation system has to detect, process and interpret the go-signal. The task-specific circuitry prepares the motor program for the required response. The response channel (Gratton *et al.*, 1988) includes all the necessary structures to execute the response as rapidly as possible. In a simple RT paradigm, the subject is given all the necessary information to plan the appropriate response before presentation of the go-signal. The task-specific circuitry can be completed well in advance (intentional aspects of motor set) and thereafter the prepared motor program has to be held in memory until transferred

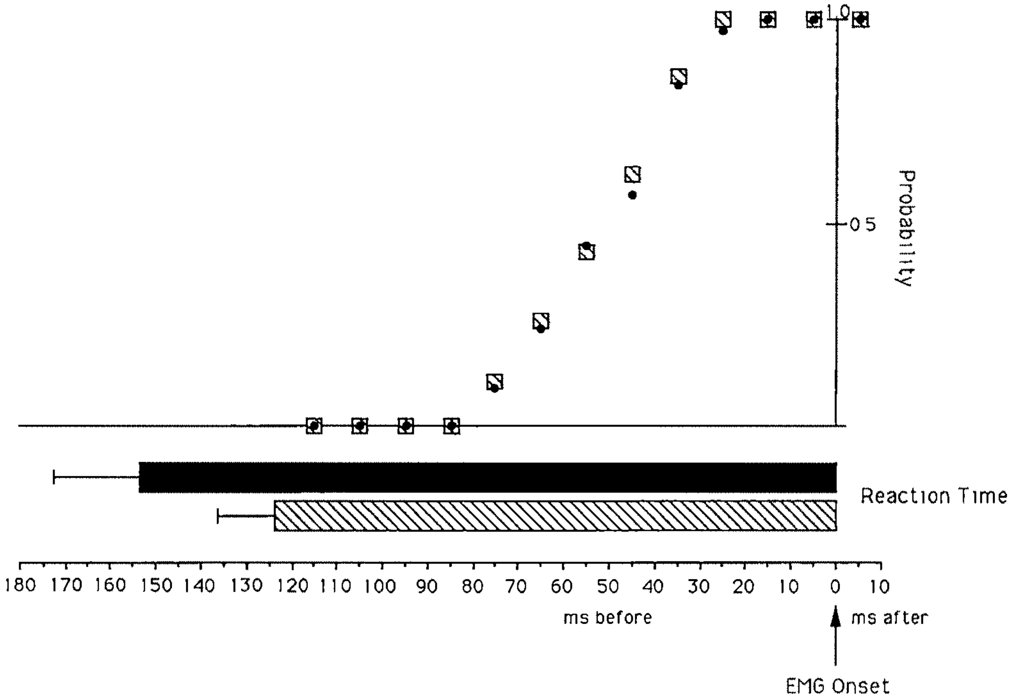

FIG. 7. Probability of evoking an MEP in the APB as a function of the interval between probing stimulus S and EMG onset Symbols for go-signals. closed circles = visual; hatched squares = visual and TMS. The bar graph shows RT (mean ± SD) plotted from EMG onset for control trials with visual go-signal (closed) and test trials with visual plus TMS go-signal (hatched). Note the significant difference in RT between the two conditions ($P < 0.001$), and the absence of differences in the probability curves (excitability increase of the motor cortex).

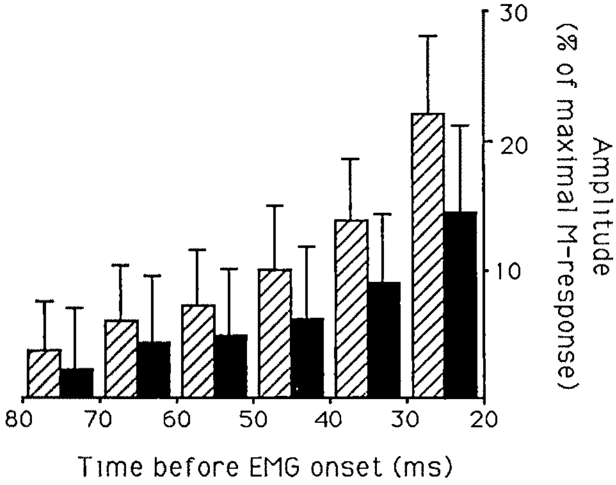

FIG. 8. Amplitudes of MEPs (mean ± SD) evoked in the APB by the probing stimulus S as a function of the interval between S and EMG onset. When the S-EMG onset interval was <20 ms the MEPs were evoked after onset of the voluntary movement and coincided with ongoing EMG activity. Therefore, the exact amplitude of the MEPs could not be determined. Columns represent trials with visual go-signal (closed) and trials with visual plus TMS go-signal (hatched).

to the response channel for response execution. In a warned RT paradigm, the stimulus evaluation system can prepare to detect the go-signal (attentional aspects of set) in response to the warning signal. Therefore, in a warned, simple RT paradigm, such as the one used in this study, the stimulus evaluation system, the task-specific circuitry and the response channel are unlikely to be activated serially (Coles *et al.*, 1985). Rather, activation of the task-specific circuitry may begin even before the warning signal, and the task-specific circuitry and the stimulus evaluation system may be active in parallel during the foreperiod (Fig. 9). Identification of a stimulus as the go-signal by the stimulus evaluation system is completed in the 'time for recognition' and sets in motion a process by which the motor program prepared and held in memory by the task-specific circuitry, is transferred to the response channel ('time for initiation'). Thereafter, the response channel needs a period of time ('time of development') to execute the response (Fig. 9).

Reaction time can therefore be divided into three periods: time for recognition, time for initiation and time of development. We think of these periods as being completed serially (Fig. 9); shortening of any of them could lead to a shorter RT.

Shortening RT by TMS: time for recognition

We define time for recognition as the time necessary for identification of a stimulus as the go-signal. This process may not require conscious recognition or perception of the go-signal (Taylor and McCloskey, 1990). The duration of the time for recognition will vary for different go-signal modalities. Therefore, this process is the most likely to be responsible for the different RTs to visual, somatosensory and auditory stimuli (Luce, 1986; Pascual-Leone *et al.*, 1992).

The shortening of RT by TMS is unlikely to be due to influences on the time for recognition. The introduction of TMS added complexity to the task of the stimulus evaluation system since the subjects had to differentiate the proper go-signal from the irrelevant TMS stimulus; failure to do so was penalized in the catch trials. Therefore, the prediction would be that the time for recognition and thus RT should have been prolonged rather than shortened (Luce, 1986).

Transcranial magnetic stimulation shortened RT by the same amount of time (approximately 30 ms) regardless of the go-signal modality, while, as mentioned above, the duration of the time for recognition depends on the go-signal modality. However, TMS could have an alerting or attention-focusing effect on the stimulus evaluation system. The role for such an effect would be limited with a warning signal, as used in our study. Nevertheless, such effects have been proposed to explain the phenomenon of 'intersensory facilitation' (Bernstein *et al.*, 1969; Nickerson, 1973). Intersensory facilitation refers to shortening of RT when a second signal to which the subject is not supposed to react is given in close proximity to the go-signal, and may shorten RT by 20–50 ms (Nickerson, 1973). However, TMS shortened RT only when it was delivered over the motor cortex, and not over frontal or parietal areas. Perception of TMS is similar regardless of the exact site of stimulation. Therefore, this topographic difference would argue against intersensory facilitation. Furthermore, effects of intersensory facilitation are more prominent if the second signal is of high intensity and of a different modality than the go-signal. Transcranial magnetic stimulation is associated with an auditory click and somatosensory scalp stimulation, both of which are proportional to the stimulus intensity. Therefore, if due to intersensory facilitation, the effects of TMS on RT should

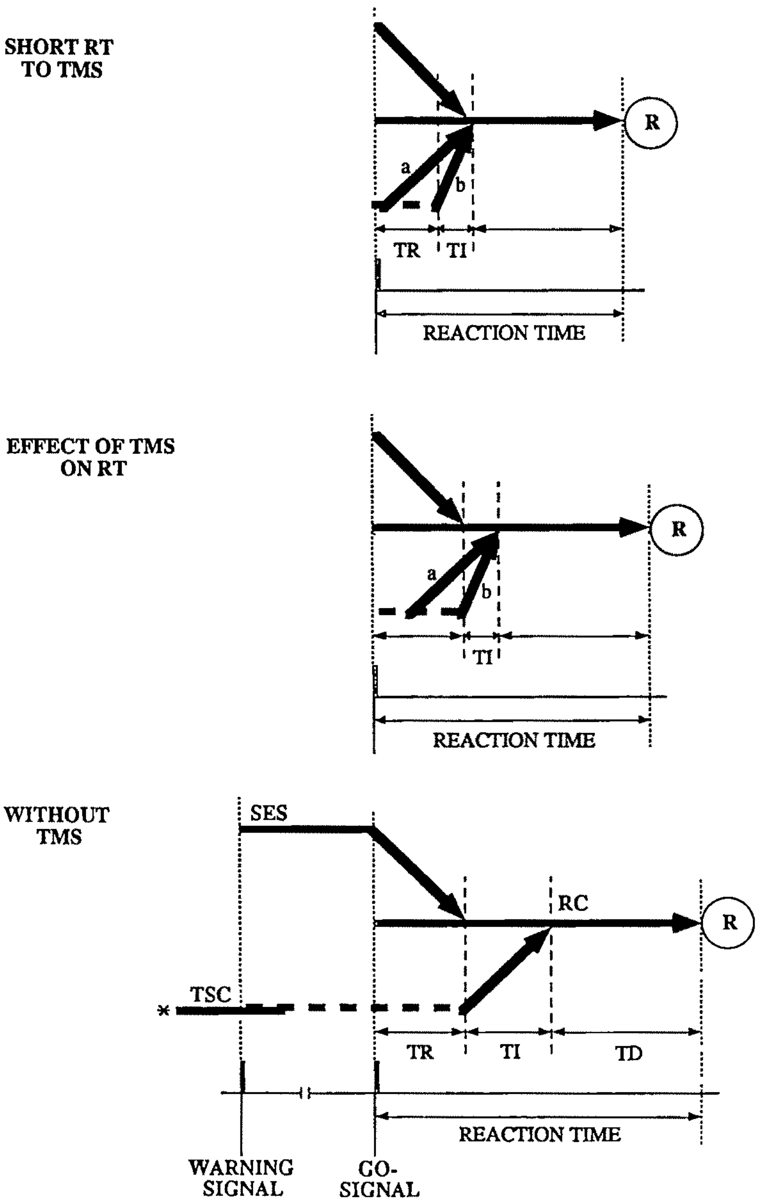

FIG. 9. Theoretical model of the processes completed during response preparation and effects of TMS on RT. SES = stimulus evaluation system, TSC = task specific circuitry, RC = response channel, R = response, TR = time for recognition, TI = time for initiation, TD = time of development. a and b represent two possible mechanisms (*see* text for details). The stimulus evaluation system has to identify the stimulus as the go-signal and is divided into attentional aspects of set (during the foreperiod) and time for recognition. The task-specific circuitry prepares the motor program for the required response and holds it in memory (dashed line) until transfer to the response channel during the time for initiation. In a simple RT paradigm, preparation of the motor program may begin or even be completed well in advance of the warning signal (*). The response channel includes all the necessary structures to execute the response as rapidly as possible as soon as the go-signal is identified (time for recognition) and the motor program is transferred from the task specific circuitry (time for initiation). Thereafter, the response channel needs a period of time (time of development) to execute the response.

be most prominent in trials using a visual go-signal and high TMS intensity. In fact, TMS at high intensities prolonged rather than shortened RT, and the effects were the same regardless of the go-signal modality.

Shortening RT by TMS: time of development

We think of time of development as an 'energizing' phenomenon (Requin, 1985) by which the motor system excitability is increased and response execution occurs when a particular threshold level is reached (Gratton *et al.*, 1988). Time of development is marked by an increase in firing rate of movement onset-related neurons in primary motor cortex (M1) which begins approximately 70–100 ms before movement onset and is directly linked to the EMG activity (Evarts, 1966, 1986; Luschei *et al.*, 1968; Fetz and Baker, 1973; Evarts and Tanji, 1976; Fetz and Cheney, 1980; Godschalk *et al.*, 1981). Transcranial motor cortex stimulation can be used to probe this period of pre-movement excitability increase. Starr *et al.* (1988) demonstrated that subthreshold stimuli, unable to evoke a MEP at rest, will produce a response when delivered ≤80 ms before EMG onset. There is an increase in the probability of evoking an MEP and in the probability of increasing the MEP amplitude when the interval is shorter between transcranial stimulus and EMG onset. This facilitation seems temporally related to the negative motor potential that precedes movement onset by approximately 20–50 ms and seems to originate from the motor cortex contralateral to the movement (Arezzo and Vaughan, 1980; Barrett *et al.*, 1985; Tarkka and Hallett, 1990), as well as to the facilitation of the H-reflex 50–100 ms before voluntary movement (Gurfinkel and Pal'tsev, 1965).

Our motor cortex excitability experiments demonstrate the absence of effects of TMS on the time of development. In accordance with Starr *et al.* (1988) and Tomberg and Caramia (1991) we found a pre-movement motor cortex excitability build-up beginning approximately 80 ms before voluntary EMG onset. The presence or absence of TMS in the go-signal affected RT, but it did not change the course of pre-movement facilitation.

Shortening RT by TMS: time for initiation

We define time for initiation as the time necessary for the transfer of the motor program generated and held in memory by the task-specific circuitry to the response channel. We hypothesize that TMS shortens RT primarily by influencing this period. Since the RT is shortened by the same 30 ms regardless of modality, the effect is likely to be on a process separate from those that are modality dependent. Transcranial magnetic stimulation could induce an earlier transfer of the motor program from the task specific circuitry to the response channel or it could speed up the transfer (Fig. 9). In the former mechanism, the transfer process would have to begin prior to stimulus recognition. In the latter mechanism, the two processes could remain serial. We favour the shift in timing of the transfer process since it provides a simpler explanation for the effects of TMS on choice paradigms in which focal subthreshold TMS to the motor cortex leads to faster response times at the cost of biasing the responses towards the contralateral hand (Brasil-Neto *et al.*, 1992). In such a condition, the completion of the transfer process prior to stimulus recognition would account for the inappropriate responses.

The topographic specificity of the effects of TMS on RT suggest that during the time for initiation the motor program is transferred to the M1. Drawing from their study on the activity of M1 neurons during foreperiod and RT prior to movement onset, Lecas *et al.* (1986) concluded: 'The features and behavioral significance of the preparation-related neuronal activity . . . suggest that M1 is only a target for a highly integrated process that progressively develops elsewhere and is triggered by the stimulus after acquiring its warning significance.' Several animal studies (Evarts, 1966, 1986; Luschei *et al.*, 1968; Fetz and Baker, 1973; Evarts and Tanji, 1976; Fetz and Cheney, 1980; Godschalk *et al.*, 1981) and human studies (Arezzo and Vaughan, 1980; Tarkka and Hallett, 1990) support this notion. The time for initiation may then be characterized by the switch of activity from set-related neurons to movement onset-related neurons. The former are most abundant in the premotor area (Wise *et al.*, 1983; Tanji and Kurata, 1989), have many intracortical connections (Wise, 1989) and may underly the short-term storage of a planned motor output until the go-signal is perceived (Wise *et al.*, 1983; Evarts *et al.*, 1984). In contrast, movement onset-related neurons are most abundant in M1 (Tanji and Kurata, 1989) and exhibit significant correlation to movement parameters (Tanji and Kurata, 1982).

Subthreshold TMS to the motor cortex may activate cortico-cortical connections and thus enhance the information transfer between set-related neurons in the premotor cortex and movement-onset related neurons (cortico-spinal neurons) in M1. The shortening of RT by suprathreshold TMS to the motor cortex ipsilateral to the response suggests a similar effect of transcallosal connections.

Shortening RT by TMS versus short RT to TMS

The effect of TMS to shorten RT to different go-signals must be differentiated from a short RT to TMS. When subjects are asked to flex their arm rapidly in response to go-signals of different modalities, RT is shortest (87.8 ± 4.1 ms) when the go-signal is a transcranial stimulus (electrical or magnetic) of subthreshold intensity delivered over the motor cortex contralateral to the responding arm (Pascual-Leone *et al.*, 1992). In the present study, TMS shortened auditory RT to 91.6 ± 7.1 ms, which is not substantially different from the short RT to TMS (Pascual-Leone *et al.*, 1992). It is conceivable, that the short RT to TMS is a response to the auditory artefact of the discharging stimulation coil, the contraction of the scalp musculature and the stimulation of the sensory scalp receptors, accelerated by the stimulation of motor cortex. In other words, the short RT to TMS may be due to shortening of the time for recognition by intersensory facilitation and shortening of the time for initiation by the direct effect on the brain (Fig. 9).

The short RT to TMS (Pascual-Leone *et al.*, 1992) is only minimally longer than the time of development (approximately 87 ms versus 80 ms). This reinforces the notion that 'the transcranial stimulus could directly activate the neuronal pool responsible for response initiation in the motor cortex, thus leading to a "reflex-like" response' (Pascual-Leone *et al.*, 1992). The short RT to TMS may represent a situation where the time for recognition is bypassed. This situation may be similar to the catch trials with errors in the present study. The stimulus evaluation system had to differentiate between the go-signal and the TMS stimulus to which the subject was not supposed to respond, thus demanding a particularly long time for recognition. The discharge of the stimulation coil during TMS is associated with a loud click (Counter *et al.*, 1990). Therefore, differentiation of TMS from the auditory go-signal is particularly difficult, the time for recognition takes longer and the potential for TMS-triggered time of development without completion or the stimulus evaluation system is greater. This may explain the significantly higher number of errors in catch trials when the auditory go-signal was used.

The normal duration of the time for initiation must be approximately 30 ms. The time of development is approximately 70–80 ms long. Together they take 100–110 ms. The shortest RT without TMS is that to an auditory go-signal and is approximately 120 ms. This allows only 10 ms for the time for recognition. Reaction times to other go-signals are longer by virtue of longer time for recognition. Thus the processes that occur during the RT seem fully accounted for.

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CONCEPTUAL APRAXIA IN ALZHEIMER'S DISEASE

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SUMMARY

Theoretical models of praxis have two major components, a praxis conceptual system that includes knowledge of tool use and mechanical knowledge and a praxis production system that includes the information needed to program skilled motor acts. Because patients with Alzheimer's disease may have an impairment of the central conceptual system, we wanted to learn if they had a conceptual apraxia by testing their knowledge of the type of actions associated with tool use, their ability to associate tools with objects that receive their action, their ability to understand the mechanical nature of problems and the mechanical advantages tools may afford.

We studied 32 subjects with probable Alzheimer's disease and 32 controls by examining tool-action relationships and tool-object associations. We tested mechanical knowledge by having subjects select alternative tools and solve mechanical puzzles by developing new tools. The Alzheimer's group was subdivided into four groups based on the presence or absence of ideomotor apraxia and a lexical-semantic deficit.

Results indicated that each of the four Alzheimer's groups differed from normal controls on at least some measures of conceptual apraxia, suggesting that Alzheimer's patients do have a disturbance of the praxis conceptual system and that impairment of this system is not directly related to language impairment or ideomotor apraxia.

INTRODUCTION

Theoretical models of praxis have two major components: (i) a praxis conceptual system that includes knowledge of tool use and mechanical function, and (ii) a praxis production system that includes the information contained in action motor programs and its translation into skilled motor performance (Roy, 1983; Roy and Square, 1985). Whereas ideomotor apraxia results from an impairment of the praxis production system, a defect in the praxis conceptual system may result in what has been termed ideational apraxia (Ochipa *et al.*, 1989).

Ideational apraxia may be seen in the use of single objects (De Renzi *et al.*, 1968; De Renzi and Lucchelli, 1988; Ochipa *et al.*, 1989). In contrast to ideomotor apraxics who typically make production errors (i.e. spatial and temporal errors) in performance of transitive gestures (Heilman and Rothi, 1985), the gestural performance of ideational apraxics is characterized by content errors (Ochipa *et al.*, 1989).

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Ideomotor and ideational apraxia have been shown to be dissociable in patients with focal (left hemisphere) brain lesions (De Renzi *et al.*, 1968; De Renzi and Lucchelli, 1988); however, reports of ideational apraxia resulting from focal lesions are rare (Zangwill, 1960).

Because the term ideation apraxia has also been used to describe the disruption of the sequential organization of tasks that require multi-stage actions (Poeck, 1983), instead of using the term ideational apraxia for patients who make content errors we will use the term conceptual apraxia.

If content errors are a result of a defect in a praxis conceptual system, there may be other types of errors that would reflect deficits in this system. We therefore posited that a conceptual disorder of praxis could occur in three domains. There may be a loss of knowledge of the type of actions associated with tools or objects (tool-object action knowledge) that should result in content errors in tool use. There may be an inability to associate tools with the objects that receive their action (tool-object associative knowledge) leading to the inappropriate selection of tools for particular tasks. There may be an impairment in the ability to understand the mechanical nature of problems and the mechanical advantages that tools may afford (mechanical knowledge or intelligence), leading to an inability to solve mechanical problems and an inability to develop tools to solve mechanical problems. Knowledge of how to solve mechanical problems may be a prerequisite to selecting or developing tools to solve problems.

Apraxia is a commonly described symptom of Alzheimer's disease and is one of the clinical signals that reportedly distinguishes this and other 'cortical' dementias from those dementing illnesses involving primarily subcortical structures (Albert *et al.*, 1974). In addition, patients with Alzheimer's disease have a particular impairment of semantic memory (i.e. central conceptual system), especially early in the course of their disease (Bayles and Kaszniak, 1987). If conceptual knowledge about tools is part of this central conceptual system, one would predict that patients with dementia of the Alzheimer's type would demonstrate a conceptual apraxia. However, reports in the literature primarily describe apraxia of the ideomotor type in Alzheimer's disease patients (Foster *et al.*, 1986; Rapcsak *et al.*, 1989). The potential for selective conceptual deficits of praxis in individuals with Alzheimer's disease has not been adequately investigated.

This study was designed to learn whether patients with degenerative dementia of the Alzheimer's type have conceptual apraxia and to determine if they have impaired tool-action knowledge, tool-object knowledge or mechanical knowledge. This study has also been designed to learn whether deficits of the praxic conceptual systems can be dissociated from impairment of the linguistic semantic system and from the praxis production systems.

METHODS

Subjects

The experimental group comprised 32 individuals with a clinical diagnosis of probable Alzheimer's disease according to NINCDS-ADRDA criteria (McKhann *et al.*, 1984). Alzheimer's subjects' ages ranged from 55 yrs to 85 yrs (mean = 72.97 yrs; SD = 7.22). Educational level ranged from 8 yrs to 17 yrs (mean = 13.13 yrs; SD = 2.89). There were 18 females and 14 males. An equal number of neurological intact adults matched for age, sex and educational level served as a control group. All subjects were right handed with no previous history of neurological or psychiatric disease.

Screening measures and subject subgroupings

Mental status. The Mini Mental State Examination (Folstein *et al.*, 1975) was used as a measure of dementia severity. Scores for the Alzheimer's subjects on this screening test ranged from 4 to 23 (mean = 13.5).

Problem-solving task. To rule out a profound disorder of problem-solving or an inability to develop an abstract attitude, each subject was given a simple problem-solving task. First, they were presented with a stacking rings set and were required to correctly stack the rings on a peg according to size. When an error was made, the correct solution was demonstrated, and the subject was required to repeat the solution. Each subject was then presented with six stacking cups and was required to correctly stack them according to size. Subjects who could not solve these tasks after two demonstrations were considered to have a profound disorder in problem-solving and were excluded from the study.

Apperceptive agnosia task. To eliminate the possibility that an apperceptive agnosia could masquerade as conceptual apraxia, subjects were presented with 10 plaster molds and were required to select one of five tools to match the mold. Only subjects who scored within two standard deviations of the controls on this screening measure (eight or more correct) were included in the study.

Ideomotor praxis test. The Florida Apraxia Screening Test (Rothi and Heilman, 1984) was administered to determine the presence of an ideomotor apraxia. This 15-item test consists of 12 transitive and three intransitive gestures that the subjects were required to imitate. Subject performances were videotaped and scored by a panel of three trained judges. Control group performance ranged from nine to 15 correct (mean = 13.4; SD = 1.62). Therefore, a score of eight or less on this test was considered to be indicative of an ideomotor apraxia.

Semantic language test. Lesser's (1974) Semantic Test was used as a measure of lexical/semantic integrity. This test of single word auditory comprehension requires the selection of a target picture from an array of four pictures, including three semantic foils. Control group performance on this measure ranged from 26 to 30 correct (mean = 28; SD = 1.28). A score of 25 or less on this measure was considered to be indicative of a semantic language deficit.

Subject subgroupings. Alzheimer's subjects were divided into four subgroups based on their performance on the above praxis and language screening measures. There were four experimental subgroups: good ideomotor praxis/no semantic language impairment (P+L+) (n = 9); poor ideomotor praxis/no semantic language impairment (P−L+) (n = 6); good ideomotor praxis/semantic language impairment (P+L−) (n = 8); poor ideomotor praxis/semantic language impairment (P−L−) (n = 9). The control group was considered a fifth group for between-group comparisons. There were no significant differences among the five groups in age [$F(4,59) = 1.07$, $P = 0.38$] or educational level [$F(4,59) = 1.74$, $P > 0.15$].

EXPERIMENTAL PROCEDURES

All subjects were administered the following experimental tasks designed to detect the presence of a conceptual apraxia. The order of experiment presentation was counterbalanced across subjects. Two demonstration trials were performed prior to test trials for each experiment. Both instructions and accuracy feedback were given nonverbally.

Experiment 1. Tool-object action relationships

If conceptual apraxia is a disorder of the conceptual praxis system, one would expect to see content errors when tools are actually being used. To assess for content errors, 12 common tools and their corresponding objects were selected (two demonstration and 10 test items). A tool was defined as an implement for performing or facilitating mechanical operations, such as a screwdriver. An object was defined as a thing to which mechanical action is directed, such as a screw. This experiment was divided into three parts: demonstration of tool use when holding the actual tool; demonstration of tool use with only the object present; demonstration of tool use with both the tool and object present. The three tasks (tool, object, and tool and object) were administered in a counterbalanced fashion with a 5-min rest between tasks. A performance was considered to have the correct content if the subject made a motion demonstrating knowledge of the type of action used with a particular tool. The following responses were considered to be content errors: tools not used or unrecognizable movement (no content); movement incorrect for tool but correct for another tool (related or unrelated content error). Production errors (i.e. content of movement recognizable and correct but poorly performed) and perseverative errors (i.e. movement to new stimulus is the same as that produced to prior stimulus) were not considered incorrect performances for this analysis. Data were analysed with an analysis of variance procedure. The within-subject factor was task (tool, object, tool and object), the between-subject factor was group, and the dependent variable was the number of content errors.

Experiment 2. Tool-object associative relationships

The appropriate use of tools in natural contexts requires intact knowledge of tool-object associative relationships. For example, to correctly perform a task such as pounding a nail, one must be able to select the correct tool to accomplish that goal (i.e. hammer). This experiment, hereafter referred to as the *tool selection task*, required that the subject select the appropriate tool to complete a task. The stimuli consisted of 12 common tools (two demonstration and 10 test items) and 12 corresponding partially completed tasks (e.g. a nail that was partially driven into a board). Each partially completed task was presented one at a time adjacent to a corresponding completed version that served to identify the goal of the task. Subjects were required to select the correct tool out of an array of five for task completion. Responses were scored on a binary pass/fail basis. An analysis of variance was performed with number of errors the dependent variable and group the between-subject factor.

Experiment 3. Mechanical knowledge

Two aspects of mechanical knowledge were investigated. The first aspect involved the ability to determine what kind of action is needed when presented with a task where associative tool-object knowledge cannot be used to problem-solve. That is, if the appropriate tool was not available to perform a required action, could the subject select a tool that shared the crucial attribute(s) of the appropriate one. This task will be referred to as the *alternative tool selection task*. It is possible that subjects could have relied on purely perceptual strategies in this task. Therefore, the second aspect of mechanical knowledge investigated was the ability to devise tools that provide the desired mechanical advantage to solve a problem. To correctly perform such a task, subjects needed to understand the goal of the task, the type of mechanical action needed for its performance and what type of tool may assist in performing the task.

Experiment 3A. Alternative tool selection. As in experiment 1, subjects were presented with partially completed tasks and a choice of five tools. There were 20 test trials. Ten of the trials were control tasks where the choice of possible tools included the proper tool for task completion (e.g. hammer and nail). After each control item, the corresponding experimental item (e.g. nail) that did not include the proper tool (e.g. hammer) was presented. For both the control and experimental items, the subjects was required to choose the tool that would best complete the task (e.g. pliers). The selection of an incorrect tool in the experimental task was scored as an error (e.g. needle).

Experiment 3B. Solving mechanical puzzles and developing new tools. The stimuli consisted of 10 sets of mechanical puzzles having as their goal the retrieval of a wooden cube (*see* Fig. 1). Each puzzle set required a different mechanical action (e.g. pushing, lifting, etc.). Each puzzle had two versions. One version was designed so that the puzzle could be solved by using one's fingers (finger puzzle). The second version was designed so that it could not be solved by using one's fingers alone and instead required a tool for its solution (tool puzzle). Subjects were presented with a $3\times3\times3$ cm metal block with a fixed bendable wire protruding from two sides. These metal blocks were to be used to devise tools.

The finger puzzle was always presented prior to its corresponding tool version. If the finger puzzle was not solved within 5 min, the solution was demonstrated. Subjects were given up to five trials to solve each finger puzzle before the corresponding tool puzzle was presented. When a tool puzzle was presented, a fresh, unbent tool was placed in the subject's hand. The subject was given 5 min to solve each tool puzzle. If a subject was unable to successfully solve a tool puzzle, the correct solution was demonstrated before the next puzzle set was presented.

An analysis of variance procedure was performed with the dependent variable being error scores for the tool puzzle task and the between-subject factor being group. In addition, t tests were conducted to determine if there were significant differences between mean scores for the tool puzzle and finger puzzle tasks within each group. Finally, an analysis of variance procedure was conducted to determine if there were between-group differences in the pattern of difference scores for solving finger puzzles versus tool puzzles.

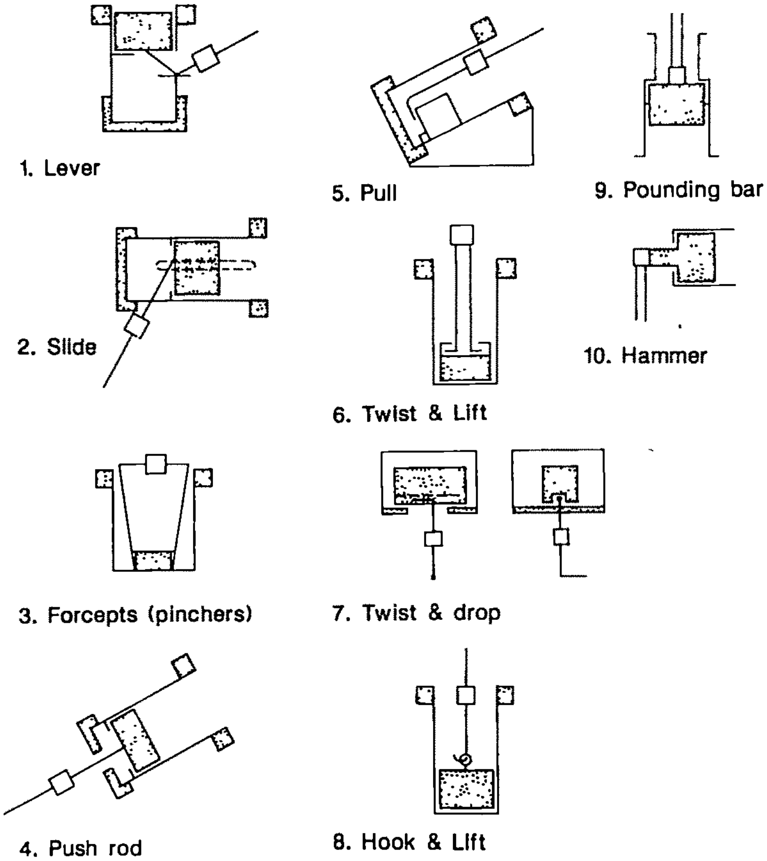

FIG. 1. Ten sets of mechanical puzzles having as their goal the retrieval of a cube. There are two versions of this task; one that can be solved by using one's fingers and the other (shown here) which requires making and using a tool.

RESULTS

Disruption of the praxis conceptual system was theorized to potentially occur in three domains: tool-object action knowledge (tool, object, and tool and object tasks), tool-object associative knowledge (tool selection task) and mechanical knowledge (alternative tool selection and tool puzzle tasks). Because each of the experimental tasks was designed to detect disruption in the conceptual praxis system, performance significantly below that of the normal control group on any one of these tasks was considered to be indicative of a conceptual apraxia.

Using a one-way analysis of variance, the means for the five groups were compared for each of the conceptual apraxia tasks (tool, object, tool and object, tool selection, alternative tool selection and tool puzzles). For each of these tasks, there was a significant between-group difference, as shown in Table 1.

Because significant group differences were found for all of the conceptual apraxia tasks, follow-up analyses were conducted using Dunnett's procedure to determine where

TABLE 1 OVERALL ANOVA FOR FIVE GROUPS ON IDEATIONAL APRAXIA TASKS

	ANOVA	
Task	*F(4,59)*	*P*
Tool	24.68	0.0001*
Object	53.53	0.0001*
Tool and object	9.28	0.0001*
Tool selection	22.66	0.0001*
Alternative tool selection	15.97	0.0001*
Tool puzzle	26.58	0.0001*

*Statistically significant

the differences existed among the five groups. Results of the follow-up analysis are shown in Fig. 2. Significant differences were found for each of the four Alzheimer's groups when compared with the normal controls on the following tasks: object, tool selection and tool puzzles. For the tool task and the alternative tool selection task, significant differences were found between the control group and Groups P+L−, P−L+

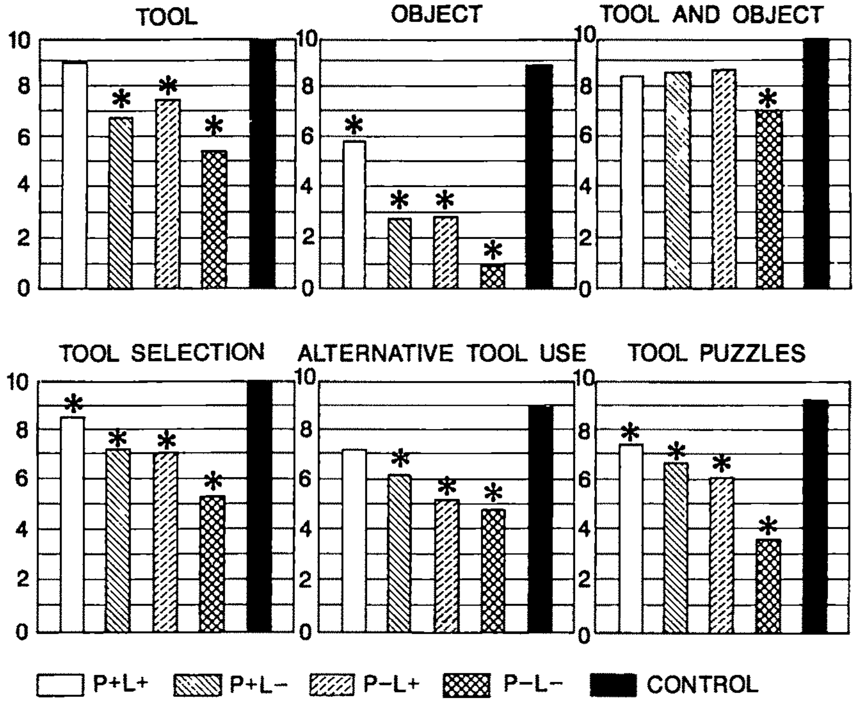

FIG. 2. Results of five subject groups on six experiment tasks. Subject groups include subjects with good praxis and no semantic language impairment (P+L+), subjects with good praxis and semantic language impairment (P+L−), subjects with poor praxis and no semantic language impairment (P−L+) and subjects with poor praxis and semantic language impairment (P−L−) *Significantly different from controls.

and P−L−. For the tool and object task, only Group P−L− significantly differed from the controls. In summary, each of the four Alzheimer's groups differed from the normal controls on at least some measures of conceptual apraxia, suggesting that this population does exhibit an impairment of the praxis conceptual system.

As seen in Fig. 2, Group P+L+ (good praxis/no semantic language impairment) was significantly impaired compared with the normal controls on three of the tasks: object, tool selection and tool puzzles. Therefore, deficits of conceptual praxis may occur in the absence of ideomotor apraxia and semantic language impairment as measured by the Semantic Test (Lesser, 1974). To evaluate further the nature of these differences, mean performances for the four Alzheimer's groups (excluding the normal controls) were compared for each of the experimental tasks. Significant differences were found among the four groups on the tool, object, tool selection and tool puzzle tasks. Results of the overall ANOVA for the four Alzheimer's groups are given in Table 2.

TABLE 2. OVERALL ANOVA FOR THE FOUR ALZHEIMER'S GROUPS ON IDEATIONAL APRAXIA TASKS

	ANOVA	
Task	*F(3,28)*	*P*
Tool	5.15	0.0058*
Object	8.43	0.0004*
Tool and object	2.38	0.0904
Tool selection	3.78	0.0215*
Alternative tool selection	2.21	0.1089
Tool puzzles	4.88	0.0075*

*Statistically significant.

The Bonferroni *t* test was used to determine where the significant differences existed among the four groups. Results of this analysis indicated that the mean performance of Group P−L− was significantly below that of Group P+L+ for the following tasks: tool, object, tool selection and tool puzzles. In addition, Group P−L+ was significantly more impaired than the P+L+ group on the object task.

A two-way factorial analysis was conducted to assess the influence of language and ideomotor praxis on the performance of conceptual apraxia tasks, as shown in Table 3. This analysis combines the two groups with good praxis and compares their mean performance on the experimental tasks with that of the ideomotor apraxics. Likewise, the two groups without detectable semantic language impairment are compared with the two groups with semantic language impairment. These results indicated there was a significant main effect of praxis and a significant main effect of language for the following tasks: tool, object, tool selection and tool puzzles. For the alternative tool task, there was only a main effect of praxis. A significant interaction between language and praxis was not found for any of the ideational tasks. As can be seen in comparison of the group means in Fig. 2, in general, the two groups with good ideomotor praxis achieved higher mean scores than the ideomotor apraxics, and the two groups with no semantic language deficit achieved higher mean scores than the groups with semantic language impairment.

TABLE 3. RESULTS OF TWO-WAY FACTORIAL ANALYSIS OF LANGUAGE AND PRAXIS EFFECTS

Task	*F(1,28)*	*P*
Tool		
Praxis	6.11	0.0198*
Language	9.35	0.0047*
Praxis × language	0.00	0.9535
Object		
Praxis	13.94	0 0009*
Language	10.91	0.0026*
Praxis × language	0.45	0.5090
Tool and object		
Praxis	0.63	0.4351
Language	0.83	0.3692
Praxis × language	1.18	0 2875
Tool selection		
Praxis	6.42	0.0172*
Language	4.75	0.0379*
Praxis × language	0.17	0.6835
Alternative tool		
Praxis	5.78	0 0231*
Language	0.69	0 4123
Praxis × language	0.16	0.6887
Tool puzzles		
Praxis	8.97	0 0057*
Language	4.60	0.0407*
Praxis × language	1.07	0.3109

*Statistically significant

In summary, conceptual apraxia can be differentiated from both ideomotor apraxia and semantic language deficits because Group P+L+ (good praxis/no semantic language deficit) performed significantly below the normal control group on three of the conceptual apraxia tasks. Furthermore, when the mean scores of the four Alzheimer's groups were compared, there were no significant differences between Group P+L− and Group P−L+. Within the Alzheimer's subjects, significant between-group differences were due to the effect of language or praxis, but not due to an interaction of these two factors.

An additional analysis was conducted to elucidate the nature of the impairment of mechanical knowledge in the Alzheimer's patients. In this experiment, the finger puzzles may be considered a control task for the tool puzzles. For this analysis, the contrast variable tool puzzle-finger puzzle was created. This contrast variable was used to assess whether the pattern of difference between the tool puzzle task and the finger puzzle task differed across the five groups. Although all five groups had significantly lower scores on tool puzzles compared with finger puzzles, there was a significant between-group difference in the magnitude of that difference [$F(4,59) = 11.02$, $P < 0.0001$]. Specifically, the differential performance on tool puzzles versus finger puzzles was greater for each of the Alzheimer's groups compared with the normal controls (*see* Table 4).

TABLE 4 COMPARISON OF FOUR ALZHEIMER'S GROUPS WITH NORMAL CONTROLS ON TOOL PUZZLES VERSUS FINGER PUZZLES

Group	*Mean difference*	*t*	*P*
P+L+	1.028	2.53	<0.05
P+L−	1.204	2.61	<0.05
P−L+	1.070	3.52	<0.01
P−L−	1.021	6.07	$\leqslant 0.01$

SEM = 1.163; *P*-values are two-tailed.

DISCUSSION

Each of the four Alzheimer's groups differed from normal controls on at least some measures, suggesting that Alzheimer's disease patients do appear to have an impairment of the praxis conceptual system. In addition, Alzheimer's subjects were found to be significantly impaired compared with normal controls at all three of the proposed domains of the praxis conceptual system (tool-object action knowledge, tool-object associative knowledge and mechanical knowledge). At this time, it is unknown if these three components of the praxis conceptual system are truly functionally distinct and how they are organized in relation to each other. It may be that there are subtypes of conceptual apraxia. Such analysis was not within the scope of the present study.

Disruption of the central conceptual system (i.e. semantic memory) in Alzheimer's disease has been well documented in studies of linguistic function (Bayles and Kaszniak, 1987). However, the relationship between semantic language impairment and deficits of tool and mechanical knowledge has not been fully investigated. In rare instances, focal neurological lesions have resulted in a selective impairment in naming visually presented material in the context of spared pantomime abilities (Lhermitte and Beauvois, 1973; Riddoch and Humphreys, 1987). This finding has led some authors to suggest that 'action semantics' may be fractionated from other semantic information (Shallice, 1987). However, the existence of a conceptual praxis system (i.e. action semantics) that is separable from the central conceptual system remains a controversial issue (Riddoch *et al.*, 1988). At present, the relationship between the well-documented semantic language deficits in Alzheimer's disease and possible disruption of action semantics in this population has not been described.

Whereas Rapcsak *et al.* (1989) found that ideational apraxia in Alzheimer's subjects, as defined by a serial object use task, was significantly correlated with impaired language comprehension, Schwartz *et al.* (1979) reported a patient who exhibited semantic language impairment in the context of spared actual object use. The finding of conceptual apraxia in the absence of detectable semantic language impairment in the present study suggests that dissociation in the opposite direction is also possible, lending support to the notion of a separable action semantics. Furthermore, the finding that a subgroup of Alzheimer's patients were impaired on tasks of conceptual praxis in the absence of ideomotor apraxia provides further evidence for the independence of the praxis conceptual and production systems.

The degree of which conceptual deficits of praxis were found in this sample is surprising in light of the scarcity of reports of these deficits in the Alzheimer's disease population.

Even though semantic language impairment has been reported to be an early feature of the disease (Bayles and Kaszniak, 1987), in this study, conceptual defects of praxis were found in patients who did not yet demonstrate semantic language impairment as tested by the Semantic Test (Lesser, 1974). This finding suggests that conceptual deficits of praxis often go undetected in Alzheimer's patients. Considering the potentially dangerous consequences of the loss of tool and mechanical knowledge, routine assessment of practice abilities in demented individuals, including specific evaluation of tool concepts, seems warranted.

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IDENTIFYING THE AFFERENTS INVOLVED IN MOVEMENT-INDUCED PAIN ALLEVIATION IN MAN

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SUMMARY

It has been clearly established that the perception of nociceptive stimulus decreases in intensity when movement is initiated in the part of the body to which the stimulus is applied. The pain alleviation is probably at least partly due to the activation of afferents which occurs during the movement. The present experiments were carried out with a view to investigating which groups of afferent fibres is mainly responsible for the gating of the nociceptive messages which occurs during movements. In eight volunteers we investigated the changes in the amplitude of the nociceptive leg flexion reflex (RIII) when the subjects were at rest, when they were performing active or passive ankle movements and when the spindle proprioceptive pathway was mobilized by applying vibratory stimulation to the Achilles tendon. Similar experiments were also carried out with eight other volunteers after anaesthetizing the ankle skin mechanoreceptors. This method was chosen because a clear-cut correlation is known to exist between the amplitude of the nociceptive motor reflex and the intensity of the pain perceived by the subject.

The data obtained clearly show that anaesthesia of cutaneous mechanoreceptors connected to the large diameter afferent fibres prevented the decrease in the motor response which otherwise accompanied both active and passive movements. Activation of the Ia fibre group by tendon vibration resulted on the contrary in an increase in the amplitude of the motor response. Movement-induced pain alleviation therefore does not mainly involve the activation of the Ia group of fibres.

INTRODUCTION

It has been clearly established that movement of a part of the body alters the perception of stimuli applied to the same region. Likewise the threshold at which tactile stimuli are perceived by humans is higher during voluntary movements than when the subjects are immobile (Coquery *et al.*, 1972; Garland and Angel, 1974; Angel and Malenka, 1982). Both active and passive movements can decrease the amplitude of the early components of cortical potentials evoked by electrical stimulations of the fingers (Papakostopoulos *et al.*, 1975; Rushton *et al.*, 1981; Rauch *et al.*, 1985). In addition, movement can bring about a decrease in the pain perception threshold: both active and passive abduction of the index finger have been found to increase both the tactile perception threshold and the pain threshold in response to electrical stimulation of the same finger by about 10% on average (Milne *et al.*, 1988).

The increase in the threshold intensities has not been attributed to non-specific factors such as distraction of the subjects' attention, since no changes in the threshold levels

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were recorded when the stimulation was applied distally to the moving body part (Angel and Malenka, 1982; Milne *et al.*, 1988).

Several mechanisms have been proposed to account for the increase in the tactile and nociceptive thresholds observed during movement. Some authors have suggested that a supraspinal inhibition mechanism may be involved, since central preparation for movement inhibits the transmission of messages by the somatosensory pathways (Coquery *et al.*, 1972; Dyhre-Poulsen, 1975; Starr and Cohen, 1985). Other authors have argued that the inhibition of nociceptive messages which is triggered by movement may involve the recruitment of afferent fibres connected to the slowly and quickly adapting mechanoreceptors in the various cutaneous tissue layers. Activation of the large-sized fibres is known in fact to set up a gating control on the spinal nociceptive neurons (Wall and Cronly-Dillon, 1960; Mendell, 1966; Hillman and Wall, 1969; Handwerker *et al.*, 1975; Salter and Henry, 1987).

Lastly, the control on the nociceptive fibres might be exerted by Ia or Ib fibres (Milne *et al.*, 1988) activated along with the muscle spindles and Golgi tendon receptors during active or passive movements. Now it is possible to preferentially recruit the muscle spindles by applying vibratory stimulation to the muscle tendons. The authors of several microneurographic studies have shown that the Ia primary endings of muscle spindles are highly sensitive to vibration, showing a one to one response pattern up to frequencies of 100–150 Hz (Burke *et al.*, 1976*a*,*b*; Roll and Vedel, 1982; Vedel and Roll, 1983). Vibratory stimulation moreover gives rise to illusory sensations of movement as well as to motor responses in either the vibrated or the antagonist muscle (Hagbarth and Eklund, 1968; Eklund, 1972; Goodwin *et al.*, 1972; Taylor and McCloskey, 1991). These motor responses and illusory sensations of movement, which reflect the large-scale recruitment of muscle spindles, were found to depend on the experimental conditions, and in particular on whether or not the subject was able to see the vibrated limb segment (Hagbarth, 1973; Roll *et al.*, 1980).

With a view to investigating which groups of fibres participate in the pain abatement occurring during movement, the amplitude of the nociceptive leg flexion reflex (RIII) was measured in resting subjects during active and passive ankle movements and under vibratory stimulation applied to the Achilles tendon. The amplitude of the RIII responses produced during active and passive ankle movements was also recorded after anaesthetizing the ankel skin mechanoreceptors and compared with that of the responses recorded with no anaesthesia.

This method was chosen because it has been clearly established on the basis of data on healthy subjects that the nociceptive reflex threshold is well correlated with the subjective pain threshold (Willer *et al.*, 1979; Willer, 1983; Chan and Dallaire, 1989). The same authors have demonstrated moreover that the amplitude of the RIII response is linearly correlated with the subjective pain intensity.

MATERIALS AND METHODS

Sixteen healthy subjects undergoing no medical treatment (nine men and seven women, aged between 24 yrs and 35 yrs) volunteered to take part in this study after being informed about the aims and methods according to the Helsinki convention.

Determination of the nociceptive threshold

The methods used in the present study were the same as those described previously (Willer *et al.*, 1979; Willer, 1983; Guieu *et al.*, 1992*a*,*b*). The subjects were seated in an armchair in a semi-reclining position, in a room at a constant temperature (23°C), in such a way that they were unable to see their lower limbs. The equipment included a constant current electrical stimulator, an electromyographic (EMG) recording device and a tracing table. The surface electrodes used to deliver the electrical stimulation were applied 2 cm apart to the external retromalleolar gutter opposite the sural nerve. The electrical stimulation consisted of trains of five 1 ms rectangular shocks delivered at 2 ms intervals with a total train duration of 13 ms. These trains were applied in three increasing-decreasing intensity series, at a frequency of 0.16 Hz, which meant that each intensity level was repeated six times. The reflex motor activity was recorded by means of surface electrodes placed opposite the distal third of the short part of the ipsilateral femoral biceps, which were connected to an EMG recording device.

The nociceptive threshold was defined as the mean minimum stimulation level required to trigger a reflex motor response in 100% of the trials.

Experimental procedure

After determining the RIII reflex threshold under the conditions described above, the first group of eight subjects was subdivided into four subgroups (A, B, C, D) which differed in the following experimental conditions applied to them: resting position; active ankle flexion-extension; passive ankle flexion-extension; vibratory stimulation of the Achilles tendon (Table 1). The passive ankle movement was obtained by grasping the subject's foot from the front and moving it. With all the subjects the stimulation intensity was set at 1.5 times the threshold level. Four successive series of 30 sural nerve stimulations were run in the four experimental situations described above. The vibratory tendon stimulation (frequency 100 Hz, amplitude 1 mm peak to peak) was applied with the subject's ankle slightly flexed in the dorsal direction so as to favour the occurrence of illusory sensations of movement.

With the second group of eight subjects, the dorsal skin of the foot and the skin folds over the Achilles tendon were anaesthetized using 2 ml of 1% lidocaine, 1/100 000 adrenalinized, taking care not to anaesthetize the external retromalleolar region. After the injection, the skin over the tibio-tarsial joint and that over the Achilles tendon was anaesthetized.

The same experimental procedure as that described above was used with this group.

Statistical analysis

The amplitude of the motor responses recorded in the various experimental situations was compared using Wilcoxon's test.

RESULTS

The results are summarized in Fig. 1 and Table 2. In the first group of subjects, both active and passive ankle movements were accompanied by a significant decrease in the amplitude of the reflex motor response. The decrease was more pronounced under the condition of the active movement.

TABLE 1. SUMMARY OF EXPERIMENTAL PROCEDURE

	Series no. 1	*Series no. 2*	*Series no. 3*	*Series no. 4*
Group A	At rest	Active movement	Passive movement	Vibration
Group B	Active movement	Passive movement	Vibration	At rest
Group C	Passive movement	Vibration	At rest	Active movement
Group D	Vibration	At rest	Active movement	Passive movement

With each subject, the four successive series of 30 stimulations at 1.5 times the threshold level were delivered under the experimental conditions in a different order to compensate for any effects due to the order. Eight of the 16 subjects were previously subjected to cutaneous anaesthesia of the dorsal foot and Achilles tendon region.

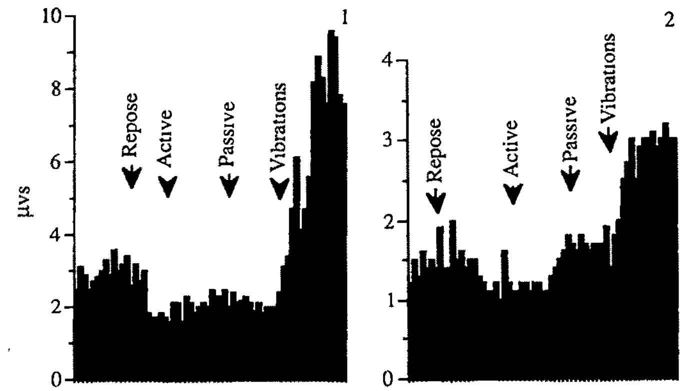

FIG 1. Changes in the amplitude of the RIII motor reflex at rest (repose), during active and passive ankle movements, and during vibratory stimulation of the Achilles tendon in two subjects from Group A. The ankle movement was accompanied by a decrease in the amplitude of the motor response, which was slightly more pronounced in the case of active movements (subject 1) Anaesthesia of the fibres connected to the cutaneous mechanoreceptors (subject 2) prevented the decrease in the amplitude of the RIII response which otherwise accompanied passive movements In both anaesthetized and non-anaesthetized subjects, the application of vibratory stimulation to the Achilles tendon resulted in a conspicuous increase in the amplitude of the motor response.

TABLE 2 SUMMARY OF STATISTICAL ANALYSES

Subjects	*Wilcoxon test*			
1	R > A, $P = 0.0002$	R > P, $P = 0.0001$	VS > R, $P = 0.0003$	P > A, $P = 0.002$
2	R > A, $P = 0.008$	R > P, $P = 0.2$, n.s.	VS > R, $P = 0.0002$	P > A, $P = 0.02$
3	R > A, $P = 0.0002$	R > P, $P = 0.0003$	VS > R, $P = 0.004$	P > A, $P = 0.0002$
4	R > A, $P = 0.002$	R > P, $P = 0.002$	VS > R, $P = 0.002$	P > A, $P = 0.0004$
5	R > A, $P = 0.0002$	R > P, $P = 0.1$, n.s.	VS > R, $P = 0.0002$	P > A, $P = 0.0002$
6	R > A, $P = 0.001$	R > P, $P = 0.0004$	VS > R, $P = 0.0002$	P > A, $P = 0.006$
7	R > A, $P = 0.0004$	R > P, $P = 0.003$	VS > R, $P = 0.001$	P > A, $P = 0.0008$
8	R > A, $P = 0.002$	R > P, $P = 0.0002$	VS > R, $P = 0.0002$	P > A, $P = 0.0002$
9	R > A, $P = 0.03$	R > P, $P = 0.07$, n.s.	VS > R, $P = 0.004$	P > A, $P = 0.0007$
10	R > A, $P = 0.04$	R > P, $P = 0.09$, n.s.	VS > R, $P = 0.0001$	P > A, $P = 0.0004$
11	R > A, $P = 0.06$, n.s.	R > P, $P = 0.003$	VS > R, $P = 0.00001$	P > A, $P = 0.0007$
12	R > A, $P = 0.001$	R > P, $P = 0.06$, n.s.	VS > R, $P = 0.0007$	P > A, $P = 0.0001$
13	R > A, $P = 0.0008$	R > P, $P = 0.1$, n.s.	VS > R, $P = 0.002$	P > A, $P = 0.00007$
14	R > A, $P = 0.008$	R > P, $P = 0.08$, n.s.	VS > R, $P = 0.001$	P > A, $P = 0.006$
15	R > A, $P = 0.006$	R > P, $P = 0.06$, n.s.	VS > R, $P = 0.002$	P > A, $P = 0.00001$
16	R > A, $P = 0.04$	R > P, $P = 0.005$	VS > R, $P = 0.0008$	P > A, $P = 0.0008$

Statistical analysis of changes in the amplitude of the RIII motor response in 16 volunteers tested in various experimental situations: at rest (R), during active (A) and passive (P) ankle movements, and during vibratory stimulation of the Achilles tendon (VS). In the first eight subjects, the amplitude of the motor response recorded at rest was significantly greater than that observed during active movements (R > A) in all eight cases, and significantly greater than that of the motor response recorded during passive movements (R > P) in six cases. In all these subjects, the passive movement had less conspicuous analgesic effects than the active movement (P > A). When the dorsal skin of the foot and the skin covering the Achilles tendon were anaesthetized (subjects 9–16), the amplitudes of the motor responses recorded at rest were not significantly higher (n.s.) than those recorded during passive movements. In all cases the amplitude of the motor response increased considerably during vibratory stimulation (VS > R).

In the second group, a decrease in the amplitude of the nociceptive reflex was observed during active movements in seven subjects. In six of the anaesthetized subjects, passive ankle movements led to no significant decrease in the amplitude of the motor response.

In all subjects, vibratory tendon stimulation resulted in a significant increase in the amplitude of the motor response. Also, all the subjects reported that the stimulation was less painful during both active and passive ankle movements. During the tendon vibration, the subjects consistently noted that the sensations elicited by electrical stimulation became more unpleasant.

DISCUSSION

The results of the present study point to the following three conclusions:

(i) Both active and passive movements were indeed accompanied by a significant decrease in the amplitude of the nociceptive motor reflex, and hence that lower pain levels were perceived by the subjects during these movements. The fact that the decrease in the pain levels was greater during active than passive movements suggests that the motor command may be involved in the movement-induced alleviation of pain. The role of the motor command does not seem to be very important here, however, since the differences between the RIII response amplitudes during active and passive movements was only slight. This confirms the results of previous studies based on the use of subjective pain threshold assessment scales (Milne *et al.*, 1988).

(ii) The activation of muscle spindles resulting from vibratory tendon stimulation (some cutaneous diffusion of the vibration was inevitable) was not accompanied by a decrease in the perceived pain levels. The movement-induced alleviation of pain observed here does not therefore seem to have involved the recruitment of Ia afferents. It might be worth studying the effects of short vibratory bursts in order to determine the post-synaptic effects of tendon vibration.

(iii) Anaesthetizing the cutaneous receptors prevented the decrease in the RIII motor response amplitude which otherwise accompanied passive movements. It is therefore via the recruitment of the large-sized afferent fibres connected to the cutaneous mechanoreceptors activated by the initiation of the movement (Hulliger *et al.*, 1979) that a gating control is carried out on the nociceptive messages. It has been reported on the basis of evoked cortical potential recordings that a control of this kind is exerted on somatosensory pathways (Papakostopoulos *et al.*, 1975; Abbruzzese *et al.*, 1981). In the present study, the nociceptive reflex therefore served to confirm objectively that a gating control is also exerted on the nociceptive pathways.

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TEXTURE DISCRIMINATION IN CARPAL TUNNEL SYNDROME

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SUMMARY

The ability to discriminate textured surfaces was measured in patients with carpal tunnel syndrome (CTS). Patients were initially diagnosed using clinical and electrophysiological criteria. The textured surfaces were gratings of alternating ridges and grooves. The gratings differed in their spatial period only, with the ratio of the ridge width to the groove width remaining constant (1:5). A two-alternative forced-choice paradigm was employed in which subjects, both patients and age-matched controls, rubbed their index finger (D2) or little finger (D5) back and forth across the surfaces. The grating spatial period at which subjects could discriminate a difference between the standard grating (spatial period = 2000 μm) and the comparison grating (spatial period in the range 2000–2900 μm) with a probability of 0.75 was taken as the measure of discriminative ability. Statistical comparison of the mean 75% values showed that: (i) when patients used D2 their discriminative ability was significantly impaired in comparison with the discriminative ability of controls using either D2 or D5; (ii) there was no significant difference in discriminative ability between patients and controls when patients used D5 to discriminate the textures; (iii) the 75% values for patients using D2 or D5 did not differ significantly. The degree of abnormality of each patient's sensory evoked potential did not allow us to predict their subsequent performance on the discrimination task.

INTRODUCTION

The carpal tunnel syndrome (CTS) results from the chronic compression of the median nerve as it passes through the carpal tunnel, a narrow space formed by the carpal bones and the flexor retinaculum. The syndrome predominantly affects middle-aged individuals and is conventionally diagnosed by a combination of clinical and electrophysiological criteria. Symptoms are initially sensory, with paraesthesiae and numbness in the distribution of the median nerve, and pain which may be felt in the hand or arm. These symptoms characteristically wax and wane and are usually more severe at night, disturbing sleep. For a full review of the clinical features of CTS, *see* Sunderland (1978).

The median nerve is easily studied using electrophysiological techniques, as the nerve trunk is in a superficial position both proximal and distal to the carpel tunnel. The ulnar nerve runs parallel to the median nerve at the wrist, but it lies outside the carpal tunnel, and thus may be used as a control. Slowing of conduction velocity and/or loss of amplitude of the sensory or motor evoked potential across the carpal tunnel segment of the median nerve is regarded as reliable evidence of CTS (Simpson, 1956; Gilliatt and Sears, 1958; Thomas, 1960; Thomas *et al.*, 1967). Marin *et al.* (1983) have reported that the sensitivity

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of these parameters in the detection of CTS is further increased following provocative manoeuvres such as flexion of the wrist.

A number of studies have been carried out to look at the pattern of sensory loss in electrophysiologically confirmed cases of CTS, using both threshold and discriminative measures of sensation. No matter which sensory measure was chosen, only a proportion of patients in these studies had impaired sensation in the distribution of the median nerve. The proportion varied between studies. For example, the reported sensitivity of vibration threshold as a test for sensory loss in CTS has varied from 4% to 87%, while the reported sensitivity of static two-point discrimination has varied from 18% to 38% (Gelberman *et al.*, 1980; Szabo *et al.*, 1984; Borg and Lindblom, 1988; Merchut *et al.*, 1990). Vibration thresholds have also been reported to increase in patients with CTS, but not in control subjects, following provocation of the CTS symptoms with wrist flexion (Borg and Lindblom, 1986).

The equivocal nature of these results throws some doubt on the usefulness of simple tactile stimuli in the assessment of sensory loss in CTS. Our aim in this study was to investigate sensory dysfunction in patients with CTS using tactile stimuli that is more complex than simple vibration or two point discrimination (Borg and Lindblom, 1988; Merchut *et al.*, 1990). We chose to use grating surfaces of alternating ridges and grooves as our test stimulus. Gratings are a simple texture and have advantages over other textured surfaces, such as sandpaper, in that they can be accurately defined by their spatial period (the distance from the leading edge of one ridge to the leading edge of the following ridge) and the ratio of the ridge width to the groove width. The spatial characteristics can be precisely varied in one dimension, thereby allowing a graded series of textured surfaces to be produced, each differing in only one spatial parameter. The ability of normal subjects to disciminate incremental changes in the spatial parameters of grating surfaces has been previously investigated (Morley *et al.*, 1983).

METHODS

Texture discrimination was studied in two groups of subjects, a patient group and a control group. The patient group consisted of 20 subjects, each of whom satisfied our criteria for CTS (*see below*). The age range of the patient group was 33–62 yrs, with a mean age of 46 yrs. Fifteen (75%) of the patients were female. Subjects included in this group were selected from patients referred to the Department of Clinical Neurophysiology, The Prince Henry Hospital, for electrodiagnostic assessment. The control group consisted of 18 subjects selected from hospital staff and the community. The age range of the control group was 26–62 yrs, with a mean age of 44 yrs. Thirteen (72%) of the control subjects were female. No hospital patients were included in the control group. All subjects gave informed consent to participate in the study.

Criteria for inclusion in patient group

For the purposes of this study, subjects were defined as having CTS, and therefore included in the patient group, if they satisfied all of the following criteria: (i) the presence of symptoms characteristic of CTS; (ii) an abnormal median nerve sensory conduction potential; (iii) an ulner nerve sensory conduction potential that was within normal limits; (iv) no history that was suggestive of peripheral neuropathy.

The symptoms considered to be characteristic of CTS were the presence of numbness, paraesthesiae or pain involving the index finger and/or the middle finger, with symptoms waking the patient at night or present on waking in the morning. Patients were not included if they described sensory symptoms in the lower limbs, of if they suffered from diseases (heavy alcohol intake, hypothyroidism, renal disease, diabetes, rheumatoid arthritis) that are associated with peripheral neuropathy.

Nerve conduction testing

As evoked sensory nerve potentials have been reported to be a more sensitive indicator of early CTS (Kemble, 1968; Kopell and Goodgold, 1968; Buchthal *et al.*, 1974; Cioni *et al.*, 1989), compound motor potentials were not used in defining CTS in the patient group.

Orthodromic sensory nerve action potentials (SNAPs) were evoked by stimulating the digital nerves of the index finger (D2) or little finger (D5) using ring electrodes wrapped around the base and the proximal phalanx of the digit. Sensory nerve action potentials were recorded at the wrist using bipolar Ag/AgCl surface electrodes placed over the median nerve (D2) or the ulnar nerve (D5). The distance between recording electrodes was 4 cm and the ground electrode was placed on the palm. Constant-voltage rectangular pulses of 0.1 ms duration were delivered at 1 Hz, with stimulus intensity suprathreshold. The surface temperature of the skin was measured at the base of D2 and at the wrist, and was maintained at $>32°$ C. Evoked potentials were averaged using a Medelec electromyograph (MS6 or MS92B). Latency was measured from the onset of the stimulus artefact to the onset of the negative phase of the SNAP. The distance between the cathode and the active (distal) recording electrode was divided by the latency to determine conduction velocity.

For the purposes of this study, the SNAP for the median nerve (orthodromic D2 to wrist) was considered to be abnormal only if the evoked potential was: (i) less than 1 μV peak-to-peak in amplitude or (ii) more than 5 m/s slower than the corresponding ulnar nerve SNAP. The SNAP for the ulnar nerve (orthodromic D5 to wrist) was judged to be within normal limits where: (i) the conduction velocity was greater than 50 m/s and (ii) the SNAP amplitude was greater than 5 μV peak-to-peak (Cioni *et al.*, 1989).

Palmar stimulation of the median nerve was also performed. In patients with CTS this technique would be expected to demonstrate a focal slowing of conduction velocity in the segment of nerve between palm and wrist. Recording electrodes were placed distally around D2 and over the median nerve at the wrist. A bipolar stimulating electrode was held in the mid-palm, with the cathode located between the first and second metacarpals. Conduction velocity from palm to finger (antidromic conduction) was compared with that from palm to wrist (orthodromic conduction). A recent study in normal subjects has shown that the conduction velocity of the two segments should be within 2 m/s (P. J. Goadsby and D. Burke, personal communication). Palmar stimulation ensured that all patients enrolled in the study were suffering from CTS and not from a median nerve injury located more distally in the hand.

Further clinical assessment of patients

A hand-held pin was used to test for impaired fingertip sensation. The patient was asked whether the sensation associated with stimulation of the fingertip of D2 and D5 felt the same. Sensation in D2 was considered abnormal if the patient reported a reduced sensation in D2. Two-point discrimination was measured on D2 and D5 using a pair of blunt calipers applied transversely across the fingerpad. The smallest distance between the caliper points for which the patient could discern the existence of two points was recorded in millimetres, and was considered abnormal if the value for D2 was greater than that for D5. Measurement of two-point discrimination was carried out as it is considered by some authors to be a sensitive test of sensory loss due to CTS (Borg and Lindblom, 1988). We compared the ability of patients to discriminate the two-point stimulus with their ability to discriminate the textured surfaces used in this study to assess the relative sensitivity of each discrimination test in detecting carpal tunnel compression. Two provocative tests were performed: Tinel's sign (paraesthesiae radiating into the hand and fingers when the median nerve was percussed gently over the wrist with a tendon hammer) and Phalen's wrist flexion text (initiating or aggravation of the patient's usual symptoms when the wrist was passively flexed for 1 min).

Assessment of control subjects

Control subjects were screened for evidence of CTS. They were included in the control group if they had: (i) no symptoms (pain or paraesthesiae) in the forearm, hand or fingers; (ii) no symptoms or disease (heavy alcohol intake, hypothyroidism, renal disease, diabetes, rheumatoid arthritis) that are associated with peripheral neuropathy; (iii) normal median and ulnar nerve SNAP.

The median nerve SNAP was considered to be normal if the conduction velocity was greater than 50 m/s and amplitude greater than 10 μV peak-to-peak.

Palmar stimulation was not performed on the control subjects.

Texture discrimination

The textured surfaces used in this study were gratings consisting of alternating ridges and grooves. The grating surfaces were 10 cm long and 4 cm wide and were manufactured from nylon using a photopolymerization technique commonly used in letterpress printing. The gratings were accurately defined by the spatial period (distance from the leading edge of one ridge to the leading edge of the following ridge) and by the ratio of the ridge width to groove width (*see* Morley *et al.*, 1983). Ten different grating sizes were employed in which the spatial period of gratings increased in 100 μm increments from 2000 μm to 2900 μm. The ridge : groove ratio remained constant at 1 : 5.

Texture discrimination in D2 and D5 was tested in the affected hand for subjects in the patient group and in the preferred hand of control subjects. Each trial consisted of a pair of grating surfaces being presented The left-hand grating was the same in each trial and had a spatial period of 2000 μm (standard) while the spatial period of the second grating of the pair (comparison) was in the range 2000–2900 μm. The comparison surfaces were presented in a pseudo-random order. Testing alternated between D2 and D5; 25 pairs of surfaces were examined using one finger, and then the other finger was tested. In most cases each pair of surfaces was presented 10 times to each finger. If the subject gave an identical response to the first five presentations of a pair, then that pair was no longer presented using that finger, thereby reducing testing time.

The discrimination task was a two-alternative forced-choice procedure. Subjects were required to rub their fingerpad, either D2 or D5, over each surface of a pair and respond *same* or *different* Subjects could use any movement profile and fingerpad force that they felt best optimized their discrimination. The number of times the subject responded *different* was counted and converted to a percentage of the total number of presentations of that particular surface pair. This percentage figure corresponds, for each pair of surfaces, to the probability of the subject giving the correct response (except for the one pair of surfaces that was not different, 2000–2000 μm).

A screen prevented the subjects seeing the surfaces and white noise from a loudspeaker masked any sound generated by the subjects rubbing their fingerpad over the grating. Each testing session lasted approximately 45 min.

RESULTS

Measurement of sensory nerve conduction velocity

Median and ulnar nerve sensory conduction velocities were measured in both patients and controls. The range of median nerve conduction velocities measured from D2 in patients was 26–55 m/s (mean 39 m/s, SD=7.4, the two patients with zero amplitude D2 SNAPs were not included) and in controls was 54–65 m/s (mean 59 m/s, SD=3.8). Ulnar nerve conduction velocities in patients and controls were very similar, with a range of 51–76 m/s in patients (mean 60 m/s, SD=6.2) and 50–67 m/s in controls (mean 57 m/s, SD=5.1). An ulnar nerve conduction velocity of 50 m/s or greater was our criterion for a normal ulnar nerve SNAP, based on the findings of Cioni *et al.* (1989). Cioni *et al.* studied 56 normal hands and found that their conduction velocities ranged from 49 m/s to 68 m/s, with a mean velocity of 55.9 m/s and SD = 4.0. This gives a mean minus 2 SDs of 47.9 m/s which compares favourably with the 20 normal hands of our study.

Palmar stimulation was performed on 18 patients. In 16 of these patients, conduction velocity from the palm to the wrist was more than 8 m/s slower than the conduction velocity from D2 to the palm, confirming that there was focal slowing of conduction across the wrist segment of the median nerve. In the remaining two patients no response could be recorded with palmar stimulation. These patients both had a very small median nerve SNAP at the wrist (<1 μV peak-to-peak).

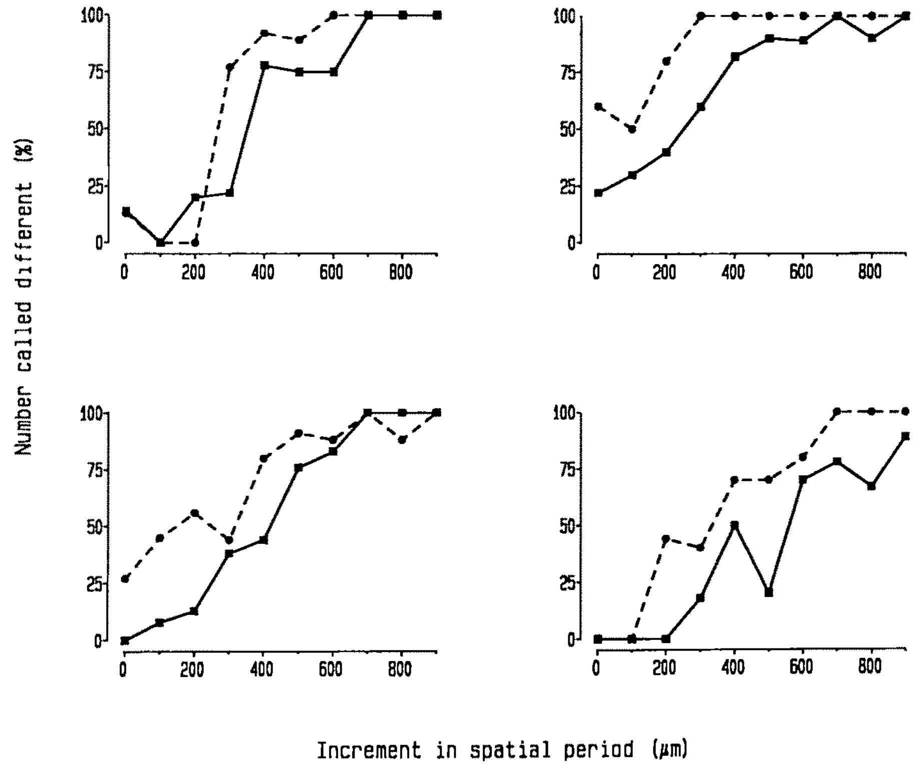

Fig. 1 Discrimination functions for four patients The number of times each comparison grating was called different from the standard grating (expressed as a percentage of the total number of presentations of that grating size) is plotted against the increment in the spatial period of the comparison grating relative to the spatial period of the standard grating (i e. 2000 µm). The discrimination function for D2 is shown by squares and a solid line and for D5 by circles and a dashed line.

Texture discrimination

Discrimination functions for four patients are shown in Fig. 1. The graphs are plots of the number of times each comparison grating was called different from the standard grating (expressed as a percentage of the total number of presentations for that comparison grating) against the increment in the spatial period of the comparison grating relative to the standard. The data for D2 are shown by squares and solid line and for D5 by circles and dashed line. The graphs for each patient show that as the comparison surface size increased there was an increase in discriminative performance. The discrimination functions for D2 are also shifted to the right relative to the functions for D5, indicating a deterioration in performance when D2 was used to discriminate the surfaces.

The mean discrimination functions for both D2 and D5 in patients is shown in Fig. 2A and for controls in Fig. 2B. The mean function for D2 of patients is shifted to the right relative to the function for D5, while for the controls there is very little difference between the discrimination functions for D2 and D5. Comparison of the D2 functions for patients and controls indicates that there is a marked difference between the discriminative

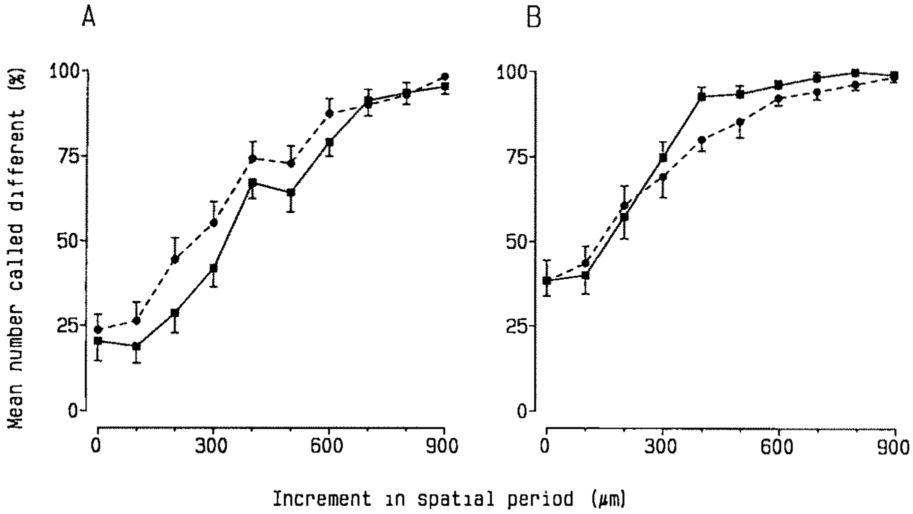

FIG. 2. Mean discrimination functions of patients (A) and controls (B). The format is the same as for Fig. 1 except that the ordinate is the mean of the number of times each comparison grating was called different from the standard grating (expressed as a percentage) for all patients or controls. Discrimination function for D2 is shown by squares and a solid line and for D5 by circles and a dashed line. Error bars are ± 1 standard error of the mean.

performance of the patients and controls, with the patients displaying an impaired performance over the majority of the range of surface sizes tested.

Measure of discriminative performance

While the discriminative behaviour of both patients and control subjects is described by the complete discrimination function, it is useful to extract a single measure that is representative of this capacity. A measure that is commonly used is the grating spatial period that yields a probability of correct response of 0.75. This represents a value halfway between a response of 50%, where the subject performs no better than chance, and 100%, where the subject can discriminate between the two surfaces without error. This value is analogous to the difference limen of the standard psychometric function (McNicol, 1972; Morley *et al.*, 1983). We calculated the 75% correct value for each subject using linear interpolation between data points on either side of 75%. One patient, who did not reach 75% correct, has not been included in the analysis.

The 75% values for patients and controls using D2 and D5 are presented in Fig. 3. The mean 75% value for patients using D2 was 2480 μm (SD = 157) and using D5 was 2424 μm (SD = 196). Mean values for controls using D2 was 2285 μm (SD = 119) and using D5 was 2333 μm (SD = 117). Statistical comparison (*t* test with Bonferroni correction; Wallenstein *et al.*, 1980) of the mean 75% values of patients and controls showed that: (i) when patients used D2 their discriminative performance was significantly worse than that of controls using either D2 ($P < 0.01$) or D5 ($P < 0.05$); (ii) the performance of the patients and controls did not differ significantly ($P > 0.05$) when both groups of subjects used D5; (iii) the 75% values for patients using D2 or D5 did not differ significantly ($P > 0.05$).

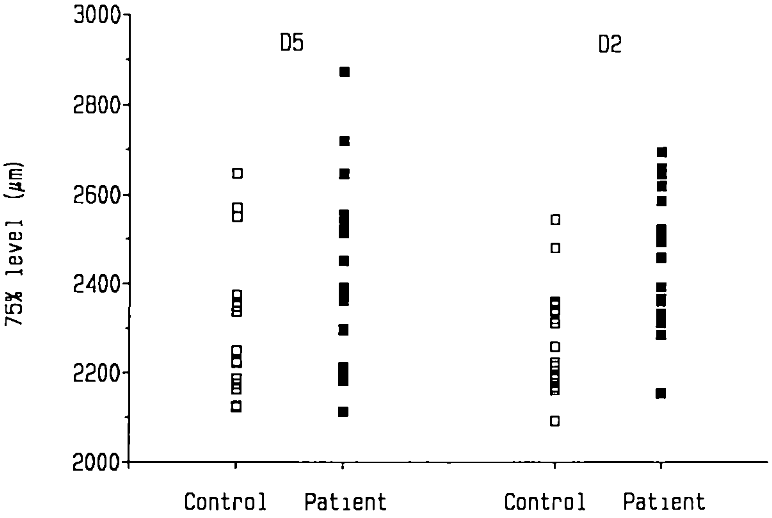

FIG. 3. The 75% values for patients (closed squares) and controls (open squares) when using D2 or D5 to discriminate the grating surfaces

Relationship between discriminative performance and electrophysiological parameters

To determine if there was any relationship between the discriminative performance of the patients and the electrophysiological parameters of conduction velocity and amplitude, the 75% values for each patient were compared with their median nerve SNAP conduction velocity and median nerve SNAP amplitude. There was found to be no correlation between the discriminative capacity of the patients and the electrophysiological parameters (correlation coefficients of 0.22 and 0.15, respectively).

In addition, we compared the 75% values with the difference between the median and ulnar nerve SNAP conduction velocity and the ratio of the median and ulnar nerve SNAP amplitudes. Again there was found to be no correlation between the discriminative capacity of the patients and the electrophysiological parameters (correlation coefficients of 0.07 and 0.30, respectively).

Relationship between discriminative performance and clinical parameters

We have also compared the discriminative performance (75% value) of each patient with their response to the four clinical tests employed (pin-prick, two-point discrimination, Tinel's sign, Phalen's wrist flexion test). The data are shown in Fig. 4. The 75% value for each patient is plotted relative to their result in each of the four clinical tests. The filled squares in each clinical test column indicate that the patient's response in that test was abnormal, while the open squares indicate the response was considered to be within normal limits. Clinical examination data were unavailable for three patients. There appeared to be no direct relationship between each patient's result on the clinical tests and their ability to discriminate the grating surfaces.

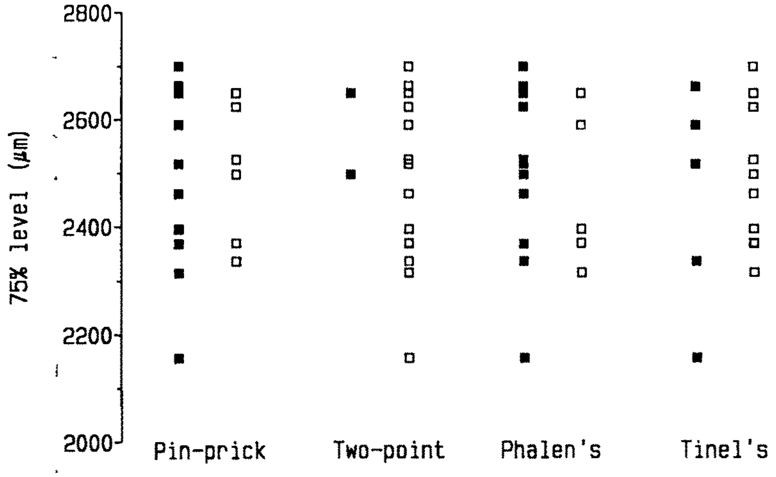

FIG. 4. Relationship between 75% values and the results of the clinical tests for each patient. The 75% value of each patient is plotted relative to their results in each of the four clinical tests. Filled squares in each test column indicate those patients who had abnormal sensation (pin-prick and two-point) or provocation of CTS symptoms (Phalen's and Tinel's). Open squares in each test column indicate those patients who had no change in sensation or no provocation of the symptoms

DISCUSSION

Measure of discriminative ability

The ability of subjects to discriminate the textured surfaces is fully described by the complete discrimination function. However, we have used the grating spatial period at which there was a 75% correct response level as a measure of the subjects' discriminative ability. This measure was derived from the discrimination functions by linear interpolation between those data points on either side of 75%. This method has the advantage of providing a measure of discriminative ability directly from the raw data. However, the derived value is sensitive to errors in these data points. In contrast, a cumulative normal curve may be fitted to the data and the 75% correct response level read from the curve directly. This method has the advantage that all data points contribute to the derived 75% value, which is therefore less sensitive to errors in individual data points. However, in a previous study (Morley *et al.*, 1983), where both methods were employed, it was found that there was little difference between the 75% values obtained in each method. We therefore chose to use the simpler linear interpolation method to derive the measure of discriminative ability.

Texture discrimination

Our data have shown that when D2 was used to discriminate grating surfaces, the patients' discriminative capacity was significantly impaired compared with the discriminative capacity of controls. However, there was no significant difference in discrimination between D2 and D5 of the patient group. This finding is surprising in that the fingerpad of D2 is innervated solely by digital branches of the median nerve, whereas D5 is innervated by digital branches of the ulnar nerve (which does not pass through the carpal tunnel). The incongruity in our results may possibly be explained through examination of the individual patients' performance (*see* Fig. 3). Consider the data for D5. The mean discrimination of the patient group was inferior to the discrimination of the control group. Yet some of the patients were able to discriminate as well as the best of the control group; it was the very poor performance of a number of patients that degraded the mean performance figure for the whole group. This observation suggests that some patients with CTS have a coexisting impairment of sensory function in D5.

A similar finding has been observed previously. Borg and Lindblom (1988) reported elevated thresholds for several sensory modalities (particularly vibration and von Frey hairs) in D5 of a proportion of their patients with CTS. Similarly, Merchut *et al.* (1990) found a number of their CTS patients had elevated thresholds in D5 for vibration and temperature. In both these studies, the normal range for the thresholds had been established from a control group. The explanation for these D5 abnormalities remains uncertain. It is possible that they are the result of a subclinical ulnar or more generalized neuropathy, although none of our patients had evidence of an ulnar neuropathy by the conventional criteria of symptoms or measurement of the ulnar nerve SNAP. Nevertheless, there is evidence of ulnar neuropathy in electrophysiological studies of patients with CTS (Sedal *et al.*, 1973; Butchthal *et al.*, 1974; Mills, 1985; Cioni *et al.*, 1989).

Mechanism underlying impaired texture discrimination in patients with CTS

The pathological changes that occur with chronic compression of the median nerve are incompletely understood, as only a small number of autopsy studies of patients with CTS have been published (Thomas and Fullerton, 1963; Neary *et al.*, 1975). The evidence suggests that, where the nerve has undergone only a mild degree of compression, the predominant abnormalities are interstitial fibrosis and demyelination without significant axonal loss. Functionally, partial demyelination of a nerve trunk will not block impulse transmission, except at sites of severe demyelination (conduction block) or at high frequencies of impulse transmission (Davis, 1972). Electrophysiologically, demyelination is characterized by marked slowing of conduction velocity, but may also result in a reduced SNAP amplitude from conduction block or dispersion of the evoked potential in the nerve. Demyelination alone should not affect detection thresholds for simple cutaneous stimuli significantly, as detection of this type of stimulus depends on only a very small number of afferents being activated (Torebjörk and Ochoa, 1980; Vallbo, 1981; Ochoa and Torebjörk, 1983). As there is a high density of mechanoreceptors in the fingertip, significant axonal loss or conduction block would have to occur before detection thresholds were affected. This is consistent with experimental findings in patients with CTS, where a large proportion of the patients have normal thresholds for simple cutaneous stimuli (Borg and Lindblom, 1988; Merchut *et al.*, 1990).

It is possible that the ability to discriminate more complex tactile stimuli (such as the gratings used in this study) is adversely affected by demyelination. Studies in the anaesthetized primate in which the activity of mechanoreceptive fibres innervating the digital skin has been recorded in response to textured surfaces moved over the fingerpad have indicated that a simple neural code based on the mean discharge rate may be used by the brain to discriminate between textures (Lamb, 1983; Goodwin and Morley, 1987). If a rate code is used by the brain to discriminate textured surfaces, then, except in cases where there is significant loss of high frequency neural transmission due to demyelination of the nerve (Davis, 1972), it is difficult to hypothesize how demyelination alone could affect texture discrimination, as a simple time average of the response of mechanoreceptive fibre populations to different grating surfaces would still be possible. However, the discharge of the different populations of mechanoreceptive fibres contains information about the grating surfaces in addition to that provided by the mean discharge rate. In particular, information about the relationship between the spatial characteristics of the grating and the speed of finger movement over the surface (temporal frequency) is represented by the temporal discharge patterns of the nerve fibres and has been implicated as also providing information possibly used in the discrimination of grating surfaces (Morley and Goodwin, 1987; Goodwin *et al.*, 1989). Demyelination could result in a greater variability in conduction velocity across the population of active nerve fibres, leading to a *blurring* of the temporal discharge pattern at higher levels of the nervous system, thereby degrading resolution.

Clinical value of texture discrimination in the diagnosis of CTS

Abnormal function of the median nerve implies that the nerve is incapable of performing its normal tasks, one of these tasks being the ability to transmit information regarding surface texture. The texture discrimination task described here is a measure of normal median nerve function, and is able to quantitatively assess impairment in one aspect of tactile perception. In comparison, sensory evoked responses (SNAPs) do not test a normal task performed by the nerve, and thus it is difficult to relate an abnormal evoked response to the subjective symptoms described by the patient.

In 20 of the patients with CTS studied, nine were outside what may be termed the normal discriminative ability, which we have defined as two standard deviations above the mean 75% correct response level for the control subjects. The severity of loss of texture discrimination was not correlated with either the amplitude loss or the slowing of conduction velocity of the sensory evoked responses from D2. Marked loss of amplitude of the SNAP or severe conduction slowing across the carpal tunnel was compatible in some patients with minimal or no impairment of texture discrimination compared with the control group, with little or no resulting disability to the patient. A similar lack of relationship between vibration and temperature thresholds and abnormality of the evoked responses has been reported by Merchut *et al.* (1990).

In clinical practice, the more abnormal the sensory evoked parameters are found to be, the more confidently can the clinician confirm the diagnosis of CTS. However, no comment about the *severity* of the CTS can be made using these parameters, if severity implies the degree of lost function that the patient is experiencing. Sensory evoked responses, although a sensitive test of median nerve compression within the carpal tunnel, are not a reliable measure of a patient's tactile disability.

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RECURRENT GUILLAIN-BARRÉ SYNDROME

CLINICAL AND LABORATORY FEATURES

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SUMMARY

The clinical and laboratory features of recurrent Guillain-Barré syndrome (RGBS) were reviewed in 12 patients in whom a total of 32 episodes fulfilled accepted criteria for Guillain-Barré syndrome (GBS). All patients were asymptomatic or only mildly symptomatic between attacks. In a given patient, the time to reach peak deficit from the onset of symptoms, the functional grade at peak deficit and the duration of the intervals between episodes varied considerably and unpredictably from one episode to the next. Analysis of these parameters across the entire group revealed no significant change as the number of attacks increased. The distribution of weakness varied between episodes with the possible exception of features of the Miller Fisher variant which were more constant. Tremor was noted in two patients and enlarged nerves in one patient. There was no evident response to immunosuppressive therapy. Results of cerebrospinal fluid (CSF) analysis and nerve conduction studies during recurrences were those expected in typical monophasic GBS. On nerve biopsy, onion bulb formations were sometimes observed after several recurrences.

The following characteristics of RGBS may be sufficiently distinctive from those of chronic relapsing polyneuropathy to justify their nosological separation: rapid onset of symptoms with subsequent complete or near complete recovery, high incidence of an antecedent illness, lack of an apparent response to immunosuppressive therapy and normal CSF protein levels at the onset of a recurrence.

INTRODUCTION

Guillain-Barré syndrome (GBS) is defined as an acute, largely reversible, idiopathic monophasic demyelinating polyradiculoneuropathy. There are several reports of patients with multiple episodes of otherwise typical GBS (Austin, 1958; Castaigne *et al.*, 1966; Pleasure *et al.*, 1968; Thomas *et al.*, 1969; Bolin and de Carle, 1972; Pollard and Selby, 1978; Seyal *et al.*, 1978; Galvan *et al.*, 1983; Wijdicks and Ropper, 1990). It has been estimated that GBS recurs in 2–5% of patients (Dyck and Arnason, 1984).

Repeated bouts of demyelinating polyneuropathy have also been described as a variant of chronic idiopathic demyelinating polyneuropathy (CIDP) (Dyck *et al.*, 1975; McCombe *et al.*, 1987), which has been referred to as chronic relapsing polyneuropathy (CRP) (Thomas *et al.*, 1969; Prineas and McCleod, 1976; Dalakas and Engel, 1981).

Although GBS and CIDP share many features, certain clinical and immunological differences may justify their separation into distinct syndromes. An antecedent viral

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infection and weakness of respiratory and facial muscles are frequent in GBS but unusual in CIDP (Dyck and Arnason, 1984). The two conditions may differ in their response to immunosuppressive treatments (Toyka *et al.*, 1988). The recent demonstration that serum of CIDP patients contains significantly fewer soluble interleukin-2 receptors than those of GBS patients further suggests the possibility of different pathogenic mechanisms (Hartung *et al.*, 1990). Differentiation of the two disorders relies on arbitrary clinical criteria: the time to reach maximum disability in GBS is usually less than 4 wks (Asbury and Cornblath, 1990) compared with more weeks or months in CIDP (Dyck *et al.*, 1975; McCombe *et al.*, 1987; Cornblath *et al.*, 1991). Hughes (1990) has defined a group of patients intermediate between GBS and CIDP, with an onset phase of 4–12 wks, as 'subacute CIDP'. The difficulty of classifying inflammatory polyneuropathies is illustrated by the occasional patient with attacks of both acute and more chronic onset. Nevertheless, for reasons outlined above and for the lack of evidence to the contrary, GBS and CIDP are generally considered different entities for clinical and research purposes.

Recurrent GBS (RGBS) and CRP, representing the respective recurrent forms of GBS and CIDP, may similarly correspond to distinct entities. However, in many descriptions of relapsing demyelinating polyneuropathies, no distinctions have been made on the basis of the time course of individual attacks and the characteristics of RGBS and CRP remain unclear (Dyck *et al.*, 1975; McCombe *et al.*, 1987). The objective of this study is to further define the features of RGBS and to compare some of these characteristics with those described in the literature for CRP.

PATIENTS AND METHODS

Recurrent GBS was defined as two or more episodes of acute idopathic demyelinating polyneuropathy each with an onset phase lasting less than 8 wks followed by complete or near-complete recovery It was felt that an onset phase of 4 wks or less may prove too restrictive for the purposes of this study Individual attacks fulfilled the clinical criteria for the diagnosis of GBS (Asbury and Cornblath, 1990).

Results of nerve conduction studies were not uniformly recorded because examinations were performed by different individuals in different laboratories over a period of more than 20 yrs. Electromyographic (EMG) criteria suggestive of demyelination were judged to recent guidelines (Asbury and Cornblath, 1990). For analysis of nerve biopsies, fascicles of the superficial and deep peroneal nerves were fixed in buffered glutaraldehyde, processed and embedded in Epon. Semithin sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and viewed in a Phillips 410 electron microscope. Samples of the nerves were teased in Epon and classified according to Dyck *et al.* (1984).

From a chart review of patients diagnosed with GBS since 1970, 12 cases conforming to the above conditions were compiled from the records of the three teaching hospitals associated with the University of Western Ontario. Over that time period, a total of 270 patients were diagnosed with GBS.

Functional grade was scored using the following scale: 0 = healthy; 1 = minor symptoms or signs and not limited in ordinary daily activities; 2 = able to walk 5 m without assistance but unable to do physical work; 3 = able to walk 5 m with assistance only; 4 = chair- or bed-bound; 5 = assisted ventilation required (Winer *et al.*, 1988).

Conventional statistical analysis was performed. Differences of sample means were evaluated by performing Student's *t* test and one-way analysis of variance was used for comparison or more than two means.

Two cases will be detailed.

Patient 1

In 1964, at age 15 yrs, this man gradually developed ascending weakness and paraesthesiae 2 wks following a rubella infection. The neurological deficit reached a peak 4 wks after onset, at which time he had severe weakness in his legs and moderate weakness in his arms. There was no facial or respiratory muscle involvement. The cerebrospinal fluid (CSF) contained 2850 mg/l of protein 10 d after onset of symptoms. He slowly improved and was discharged from the hospital 3 mths later. He fully recovered within 6 mths.

In 1967 he noticed mild paraesthesiae and weakness in his fingers and toes 2 wks following an upper respiratory tract infection (URTI). There was gradual progression of weakness over 2 wks but he remained ambulatory. He was hospitalized for 1 mth and recovered fully in 2 mths.

In 1971 he had a brief illness with malaise and vomiting followed 2 wks later by tingling in his fingers and toes. He developed ascending weakness and facial paraesthesiae over the next week. By the 10th d, he was quadriplegic, had bilateral facial weakness and required assisted ventilation. There was no ophthalmoplegia. He began to improve 3 wks after onset and was on the ventilator for a total of 3 wks. He was discharged from the hospital after 3.5 mths and recovered completely by 7 mths. He was treated with prednisone during his acute illness.

In 1977, 2 wks after a 2-d bout of malaise and vomiting, he noted paraesthesiae in his fingers and toes. He developed mild leg weakness but his arms and face were not involved. He was admitted to hospital and treated with prednisone 20 mg daily from the second day for a period of 3 wks. Recovery was again complete.

The fifth episode occurred in 1981, 2.5 wks following a 1-d episode of nausea, vomiting and anorexia. He developed paraesthesiae in his fingers and toes and mild leg weakness. The weakness gradually ascended to involve his arms, reached a peak by day 35 and began to improve 4 d later. By day 25, a mild postural and intention tremor of both hands became apparent and slowly worsened. Examination on day 43 revealed mild weakness of all four limbs, more severe distally with wasting of extensor digitorum brevis bilaterally. The tremor became prominent and affected the hands and, to a lesser degree, the legs and head. Tremor frequency was 4–5 Hz. He was areflexic. Sensory examination revealed a mild deficit to pin and vibration and reduced proprioception distally, The ulnar nerves and dorsal cutaneous nerves of the feet were enlarged. Light tapping over the ulnar, median, radial, common peroneal and the dorsal cutaneous nerves of the feet evoked a sensation of intense tingling in the cutaneous territory of those nerves. The CSF contained 1510 mg/l of protein and one leucocyte per cubic millimetre. The posterior tibial nerve conduction velocity was 11.6 m/s and conduction block was found in multiple motor nerves. Biopsy of the right deep and superficial peroneal nerves revealed acute demyelination, remyelination, onion bulb formation and mild lymphocytic infiltration (Fig. 1). He was treated with prednisone, 20 mg/day from day 3. Prednisone was slowly tapered and discontinued on day 78, at which time he felt he had recovered at least 90% of his strength. The tremor persisted but to a lesser degree. Within days after discontinuing prednisone, he noted increasing paraesthesiae and weakness. Prednisone was temporarily reinstituted; he was improved by day 98 and felt normal on day 120.

In October 1982 he noticed increasing numbness of his fingers and toes. Examination on day 5 revealed mild bilateral wasting of intrinsic foot muscles and mild distal reduction in vibration sense but no weakness. Myotactic reflexes except ankle jerks could be elicited. Treatment with prednisone, 80 mg/day, was initiated but he rapidly developed ascending weakness, facial diplegia and increasing postural and intention tremor of arms and legs. He remained ambulatory. He was treated with four plasma exchanges of 4 l each over a period of 2 wks. During plasma exchange his strength started to improve but 2 wks following the last exchange he again developed weakness which gradually started to improve 3 wks later. Despite increasing strength, the tremor worsened and was often incapacitating. He resorted to ingesting 1.5 oz rye whisky before meals which improved the tremor and facilitated eating. Propanolol was ineffective. Over the next 6 mths, his strength returned to normal and the termor almost completely disappeared. Myotatic reflexes were all normal. He went back to work full time as a builder.

The seventh episode began in 1986, 2 wks after a viral illness, when he noted paraesthesiae in his fingers and toes. Ascending weakness reached a peak on day 45, at which time he required assistance to walk. Reflexes were absent. Hand tremor slowly increased but remained relatively mild. He did not receive immunosuppressive treatment. Over the next 5 mths he fully recovered and was back at work.

Patient 2

In 1954 this 32-yr-old woman developed blurred vision, leg weakness and paraesthesiae of her hands and feet a few days after an upper respiratory tract infection. After several days, she complained of increasing gait unsteadiness and limb weakness until she could no longer walk. She had bilateral facial weakness, complained of diplopia and bilateral ptosis. Deep tendon reflexes were absent. She did not suffer significant respiratory failure. She made a gradual recovery and was functionally normal after a year.

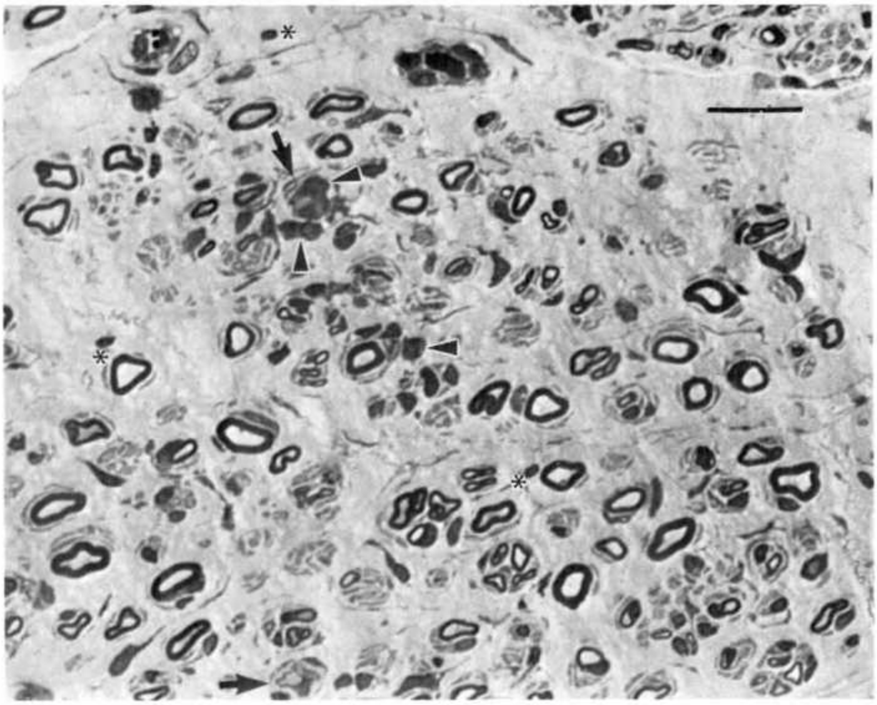

FIG. 1. Biopsy of the superficial peroneal nerve of patient 1 demonstrating inflammatory demyelination. Many axons show disproportionately thin myelin sheaths with early onion bulb formation. A few fibres are segmentally demyelinated (arrow) with associated macrophages (arrow head). Lymphocyte-like mononuclear cells (asterisk) infiltrate the endoneurium. Cross-section: 1 μm; toluidine blue; magnification = 25 μm.

In 1981, 2 d after a cold, she noticed weakness in all limbs, paraesthesiae in her hands and feet and diplopia. She was found to have mild weakness in all limbs, areflexia and weakness of abduction of the right eye. She did not receive immunosuppressive treatment. After 1 wk, the weakness and paraesthesiae gradually improved but diplopia lasted 3 mths.

One year later, 1 wk after an upper respiratory tract infection, she developed gait unsteadiness and felt bilateral leg weakness. The next day, she noted bilateral hand numbness, diplopia and could not walk. By day 6, bulbar weakness and respiratory failure necessitating ventilatory support had developed. Maximum disability was reached on day 8 when she had marked ptosis, facial weakness, complete bilateral external ophthalmoplegia, quadriplegia, areflexia and severely reduced vibration and position sense in all limbs. There were no automatic abnormalities. The first sign of improvement, bilateral eyelid movement, was noted on day 11. She breathed independently by 4 wks and walked 1 wk later. Immunosuppressive treatment was not used. At discharge from hospital, 10 wks after onset, she had mild right ptosis, mild bilateral weakness of eye abduction, minimal distal weakness, moderate generalized ataxia and reduced vibration sense distally. On day 15, CSF contained 1440 mg/l of protein and one leucocyte per cubic millimetre. By day 60, CSF protein had fallen to 490 mg/l. By 6 mths, neurological examination was normal except for mild incoordination of fingers movements, mild gait ataxia and areflexia.

RESULTS

Clinical features

Table 1 summarizes the clincal features and the course of the neurological condition in each case. Among the 12 subjects, seven were male and five were female. The average

age at the onset of the initial episode was 28.9 yrs (8–63 yrs) and at the onset of the last episode 46.1 yrs (16–69 yrs). While the inclusion criterion for the time to reach peak disability from the onset of symptoms was 8 wks, for most episodes this interval was 2 wks or less. The mean interval between attacks was 9.7 yrs (2 mths to 43 yrs). Eight patients had a single recurrence, three patients two recurrences and one partient six recurrences. An episode of isolated unilateral facial palsy in one patient (patient 8) was not included.

As only one patient had more than three attacks, statistical analysis was carried out only for the first three episodes. From the first to the third episode, there seemed to be a trend towards shorter intervals from the antecedent illness to symptom onset, towards longer intervals from symptom onset to peak deficit and towards longer intervals between episodes (Table 2). However, these changes did not reach statistical significance ($P > 0.05$). The mean functional grade at peak deficit was quite similar from one attack to the next (Table 2).

Sixty-seven percent of initial episodes and 85% of recurrent episodes were preceded by an infective illness (20 episodes in nine patients), immunization (one episode: tetanus toxoid) or were associated with pregnancy or with the immediate post-partum period. In patient 5, two episodes were associated with the first pregnancy (at seventh month of pregnancy and 4 d post-partum) and one episode began 21 d after delivery of the second child 5 yrs later. In patient 8, the first attack of GBS appeared 60 d post-partum; the other two episodes were unrelated to pregnancy. In one patient, serology suggested a recent herpes simplex infection.

In nine patients, the neurological symptoms and signs were typical of monophasic GBS. The most common initial symptoms were weakness and paraesthesiae distally in the limbs. Facial weakness was present in 47% of episodes, bulbar weakness in 30% and respiratory muscle weakness requiring assisted ventilation in 13%. All patients were areflexic. Nerves were palpably enlarged only in patient 1, following the fifth episode. Postural and intention tremor were prominent features in two patients. The characteristics of the tremor in patient 1 were outlined above. A similar slow postural and intention tremor was seen in patient 4 at the onset of the second episode. There was no clear relationship between the degree of weakness and the amplitude of tremor, except for the absence of tremor when plegic. No cerebellar or extrapyramidal signs were noted.

In three patients (patients 2, 9, 12), features of the Miller Fisher variant of GBS were prominent (Fisher, 1956). In every episode of these three patients, external ophthalmoparesis of variable severity was associated with limb weakness and, in four episodes, with respiratory failure requiring assisted ventilation. Ataxia in the second bout of patient 9 was marked and associated with only mild weakness of limb muscles. Ocular signs were reported in two other patients: mild bilateral ptosis during both episodes in one patient and weakness of both external rectus muscles during one attack in another patient. Neither presented with limb or gait ataxia.

After the initial episode, symptomatic recovery was complete in eight patients. Among the other four patients, two described mild proximal weakness, one complained of distal paraesthesiae in the feet and of gait unsteadiness and one noted mild incoordination of finger movements. No patient was limited in daily activities. After the second episode, three patients fully recovered subjectively and objectively. Five were reported to have mild residual distal sensory or motor signs with depressed ankle jerks after a follow-up

TABLE 1 CLINICAL FEATURES AND CSF PROTEIN LEVELS IN 12 PATIENTS WITH RECURRENT GBS

Case/ sex	*Age first episode*	*Age last episode*	*Episode*	*Recurrence interval (mths)*	*Antecedent condition*	*Time to onset of symptoms (days)*	*Time to peak deficit (wks)*	*Grade at peak deficit*	*CSF protein mg/l (days)*
1 M	15	37	1	—	Rubella	14	4	4	2850 (10)
			2	36	URTI	14	2	2	—
			3	48	Enteritis	14	2	5	—
			4	72	Enteritis	14	2	2	—
			5	48	Enteritis	17	7	2	1510 (15)
			6	10	Enteritis	10	3	3	—
			7	44	Enteritis	14	7	4	2850 (12)
2 F	32	59	1	—	URTI	5	1	4	—
			2	324	Flu-like	2	1	2	—
			3	14	URTI	7	1	5	490 (60)
3 M	32	46	1	—	Flu-like	25	1	3	1440 (15)
			2	175	URTI	14	1	4	1420 (7)
4 M	63	69	1	—	Herpes simplex	—	1	5	870 (3)
			2	67	Flu-like	2	1	4	1400 (19)
5 F	25	30	1	—	Pregnancy+urti	14	2	4	1900 (15)
			2	2	P-partum (4 d)	—	2	4	5750 (27)
			3	65	P-partum (21 d)	—	2	2	1320 (12)
6 F	11	16	1	—	—	—	2	3	—
			2	60	Mononucleosis	5	1	4	570 (5)
7 M	28	41	1	—	URTI	42	1	4	—
			2	156	URTI	21	1	2	364 (1)
8 F	34	47	1	—	P-partum (2 mths)	—	4	2	1000 (60)
			2	120	—	—	1	1	—
			3	36	—	—	8	2	—
9 M	44	51	1	—	—	—	1	4	840 (16)
			2	79	URTI	2	1	5	380 (3)
10 F	40	53	1	—	Tetanus toxoid	17	2	4	390 (4)
			2	156	URTI	7	1	4	300 (2)
11 M	8	51	1	—	—	—	1	4	6000 (12)
			2	516	URTI	20	1	4	539 (1)
12 M	27	54	1	—	—	—	1	4	—
			2	336	—	—	1	5	500 (10)

Case/ sex	*Episode*	*Facial weakness*	*Bulbar weakness*	*Extra-ocular muscle weakness*	*Tremor*	*Treatment*
1 M	1					
	2					
	3	+	+			Prednisone
	4					Prednisone
	5				+	Prednisone
	6	+			+	Prednisone+plasmapheresis
	7	+			+	
2 F	1	+	+	Diplopia, Ptosis		
	2			Right VI		Prednisone
	3	+	+	Total external ophthalmoplegia		
3 M	1			Bilateral VI		
	2	+	+			Prednisone
4 M	1	+	+			Prednisone
	2		+		+	Prednisone

Case/ sex	*Episode*	*Facial weakness*	*Bulbar weakness*	*Extra-ocular muscle weakness*	*Tremor*	*Treatment*
5 F	1	+				Prednisone
	2	+	+			
	3					
6 F	1					
	2	+	+			Prednisone
7 M	1	+				
	2					
8 F	1					
	2					
	3					
9 M	1			Total external ophthalmoplegia		
	2	+	+	Total external ophthalmoplegia		Plasmapheresis
10 F	1	+		Mild bilateral ptosis		
	2			Mild bilateral ptosis		Plasmapheresis
11 m	1					
	2	+				Plasmapheresis
12 M	1			Diplopia		
	2	+	+	Total external ophthalmoplegia		Plasmapheresis

TABLE 2 AVERAGE VALUES OF PARAMETERS FOR EACH EPISODE

	Episode no						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Interval from antecedent condition to onset of symptoms in days (number of patients)	19.5 (6)	9.7 (9)	10.5 (2)	14.0 (1)	17.0 (1)	10.0 (1)	14.0 (1)
Time to reach peak deficit in weeks	1.8 (12)	1.2 (12)	3.2 (4)	2.0 (1)	7.0 (1)	3.0 (1)	7.0 (1)
Grade at peak deficit	3.8 (12)	3.4 (12)	3.5 (4)	2.0 (1)	2.0 (1)	3.0 (1)	4.0 (1)
Interval between episodes in months	—	169.0 (12)	41.0 (4)	72.0 (1)	48.0 (1)	10.0 (1)	44.0 (1)

period averaging 38 mths. The other myotatic reflexes reappeared. Among the other three patients, symptomatic proximal weakness persisted in two and sensory ataxia affecting gait persisted in the other. In three of the four subjects with two or more recurrences, neurological deficits tended to accumulate with the number of recurrences but no significant disability resulted. In patient 1, no deficits accumulated and only mild vibration loss and atrophy in the feet were documented following recovery from the sixth recurrence.

Nine episodes in six subjects were treated acutely with prednisone, four episodes in four subjects with plasmapheresis and one episode with both prednisone and plasmapheresis. Because of the variable modes of therapy, variable dosage schedules and the uncontrolled nature of treatment, the possible effects of the above therapies were not analysed. It can be stated, however, that there were no instances of an apparent dramatic response to treatment.

No patient had a family history of demyelinating polyneuropathy.

Cerebrospinal fluid

Cerebrospinal fluid was obtained in all patients on at least one occasion, during a total of 21 episodes. The protein level was elevated (>450 mg/l) in the symptomatic phase in all attacks in which measurements were made 1 wk or more after the onset of symptoms: the average CSF protein level was 2480 mg/l (840–6000 mg/l; $n = 11$) and, in all cases, the leucocyte count was normal (less than 5×10^9 cells per litre). In two patients, during the early recovery period (within 60 d of peak deficit), the protein level was mildly elevated, 490 and 1000 mg/l. Protein levels in the CSF were not obtained in any subject during the asymptomatic interval. However, all protein measurements performed within 1 wk of onset of a recurrence were normal (five episodes in three subjects) or only mildly elevated (two episodes in two subjects).

Electrophysiological studies

In total, 18 nerve conduction studies were performed on 10 patients during 13 different episodes (Table 3). Only three studies were performed during the first episode and first interval (most initial episodes occurred prior to 1966), limiting the possibility of longitudinal analysis. In total, 14 nerve conduction studies were consistent with a demyelinating polyneuropathy (10 episodes in seven patients), one was normal, two showed only prolongation of the terminal latency of the median nerve and one provided insufficient evidence for multifocal demyelination. Eleven of 15 examinations performed during the acute symptomatic phase revealed evidence of demyelination. Only three examinations were performed following clinical recovery: two studies showed only mild abnormalities while the other revealed more severe demyelination (patient 8, 7 mths following the fourth episode at which time she was mildly symptomatic).

Median nerve conduction velocity during the acute phase averaged 37.4 m/s (14 studies) and following recovery 34.2 m/s (three studies). In the early days of the fifth attack of patient 1, the marked prolongation of the distal latencies from stimulation of the median and ulnar nerves at the wrist suggested very severe demyelination and continuous (non-saltatory) nerve conduction in those segments. Conduction block or temporal dispersion were present in at least one nerve in 10 of 14 examinations (five of eight patients) during the acute symptomatic phase and in two of four studies (one of three patients) during asymptomatic interval. Median, ulnar and sural sensory nerve action potentials were absent or diminished in amplitude in all but one study. Fibrillation potentials of positive sharp waves in distal muscles were reported in five of 14 needle examinations (four of nine patients) during the acute phase and in two of four examinations (one of three patients) in the asymptomatic interval.

Histological observations

Fascicular biopsies of the superficial and deep peroneal nerves (SPN and DPN) were performed in two subjects (patients 1, 2). In patient 1 (Fig. 1), the biopsies were performed during the fifth attack. Examination of both nerves under the electron microscope revealed expansion of the endoneurium by excess deposition of ground substance and collagen. The overall density of myelinated fibres was reduced. Numerous fibres were relatively thinly myelinated and showed evidence of recurring demyelination with onion bulb formation. Numerous nomonuclear cells were scattered within the endoneurium and around endoneurial blood vessels. Examination of teased fibres

TABLE 3. NERVE CONDUCTION STUDIES AND ELECTROMYOGRAPHY IN 10 RGBS PATIENTS

			Motor conduction								*Sensory conduction*			
			Median		*Ulnar*		*Common peroneal*							
Case	*Episode*	*Time from onset*	*Latency (ms)*	*Velocity (ms)*	*Latency (ms)*	*Velocity (ms)*	*Latency (ms)*	*Velocity (ms)*	*Conduction block*	*Temporal dispersion*	*Median Amplitude (uV)*	*Ulnar Amplitude (uV)*	*Sural Amplitude (uV)*	*EMG Fibs/PSW*
1	5	days	—	—	—	—	—	16.0	Y	Y	—	—	—	—
	5	days	58.0	28.0	32.0	6.3	—	—	Y	—	—	—	—	—
	5	42 d	—	21.4	—	—	—	14.0	—	—	NR	—	NR	N
2	3	16 d	3.9	46.0	—	—	4.5 F = 72.7	45.0	N	N	2.5	—	2.5	N
4	1	42 d	6.2	50.0	4.6	47.0	—	43.0	—	Y	NR	NR	NR	Y
	2	10 d	3.8	39.0	4.0	74.0	—	41.0	—	Y	NR	NR	NR	N
6	2	70 d	—	40.0	—	50.0	—	—	—	N	—	—	—	Y
7	2	yrs	4.4	46.7	5.0	45.0	5.0	45.0	N	—	NR	NR	NR	N
8	2	60 d	2.9	18.0	—	25.3	5.3	43.3	Y	Y	—	—	—	N
	4	45 d	4.9	30.0	3.2	23.8	5.3	27.0	Y	Y	5.3	—	—	Y
	4	200 d	3.6	25.8	—	—	—	—	Y	Y	—	—	—	Y
9	1	9 d	5.0	55.0	3.0	62.0	7.0	47.0	N	N	—	—	—	N
	1	300 d	4.6	57.0	2.5	65.0	—	—	N	N	—	—	NR	N
	2	10 d	4.0	35.7	3.4	57.1	—	43.0	N	N	11.0	6.0	—	Y
10	2	5 d	2.9	54.6	2.3	62.7	4.8	44.0	N	N	19.0	17.0	4.0	N
11	2	10 d	5.3	35.3	3.6	36.6	5.0	18.3	Y	N	2.0	—	2.0	Y
	2	42 d	16.2	13.2	8.8	8.1	—	—	Y	Y	—	—	—	Y
12	2	10 d	5.0	40.0	3.6	51.0	6.4	42.6	Y	Y	12.1	8.4	9.6	N

d = days, F = F-wave, NR = no response, Y = present, N = absent, Fibs/PSW = fibrillation potentials or positive sharp waves.

(Table 4) showed very marked evidence of chronic ongoing segmental demyelination. Associated axonal degeneration was minimal. On electron microscopy, numerous formerly myelinated fibres showed a lack of myelin sheath or were thinly myelinated.

In patient 2, the biopsies were performed during the third episode. Changes in both nerves were identical. Light microscopy revealed a mild reduction of myelinated fibres, particularly of large fibre size. A number of fibres were undergoing acute Wallerian degeneration. Occasional regenerative clusters were noted. Rare fibres with segmental demyelination and a few thinly myelinated axons were observed. A few macrophages were present throughout the endoneurium and in a perivascular distribution. Examination of the teased fibre preparations revealed a considerable degree of ongoing Wallerian degeneration with only very little evidence of paranodal and repaired segmental demyelination (Table 5).

Electron microscopy revealed a mild reduction of myelinated fibres and numerous fibres showing early stages of Wallerian degeneration. Only rare fibres with segmental demyelination were observed. No lymphocytic infiltration was noted.

TABLE 4 CASE 1 TEASED FIBRE ANALYSIS (%)

Nerve	*A and B*	*C*	*D*	*E*	*F*	*G*	*H*	*Total (n)*
Deep peroneal nerve	19	7.5	69.9	0.9	2.8	—	—	106
Superficial peroneal nerve	10	—	84.4	1.1	1.1	—	—	90

TABLE 5 CASE 2 TEASED FIBRE ANALYSIS (%)

Nerve	*A and B*	*C*	*D*	*E*	*F*	*G*	*H*	*Total (n)*
Deep peroneal nerve	75.4	0.9	0	15.4	8.2	—	—	110
Superficial peroneal nerve	77.5	—	0	17.4	5.2	—	—	115

DISCUSSION

Twelve patients with one to six recurrences of GBS are reported. In a given patient, the functional grade at peak deficit, the time to reach peak deficit and the time interval from the antecedent illness to the onset of neurological symptoms varied considerably and unpredictably from one episode to the next as did the duration of asymptomatic intervals between episodes. In addition, analysis of these parameters across the entire group revealed no significant change as the number of episodes increased.

Except in one patient in whom all episodes were related to pregnancy or to the early post-partum period, the nature of the antecedent illness tended to differ from episode to episode. This non-specificity in triggering events may explain the variable intervals to the onset on neurological symptoms. This contrasts with the patient described by Pollard and Selby (1978) who developed GBS on three occasions following the administration of tetanus toxoid; the interval from exposure to onset of symptoms tended to decrease with exposure.

Although the absence (nine subjects) or presence (three subjects) of ophthalmoparesis with ataxia were constant from one episode to the next in each patient, facial, bulbar and respiratory involvement varied considerably. This contrasts with previous reports of RGBS in which facial or bulbar weakness seemed consistent from one episode to the next (Castaigne *et al.*, 1966; Wijdicks and Ropper, 1990) and argues against reactivation of smouldering inflammation at sites specifically affected during the initial attack as the main mechanism of recurring polyneuritis (Asbury *et al.*, 1969; Wijdicks and Ropper, 1990). Rather, the changing distribution of weakness suggests a more generalized reactivation of the disease. Features of the Miller Fisher variant of GBS were much more constant from episode to episode in patients presenting initially with this syndrome as was reported elsewhere (Kaplan *et al.*, 1985; Schapira and Thomas, 1986; Kaiser-Smith, 1989) suggesting the possibility of dysimmune mechanisms different from those operant in recurrences of classical GBS.

In two of our patients (patient 1, episodes 5, 6, 7; patient 4, episode 2) a postural and intention tremor was prominent. The characteristics of this tremor were very similar to those described by Dalakas *et al.* (1984) in seven patients with CRP. We also observed that tremor was not associated with cerebellar or central nervous system signs and that it correlated poorly with the degree of weakness and loss of proprioception. The above authors noted that tremor developed preferentially during later relapses of CRP. Similarly, tremor is unusual in monophasic GBS but has been reported during recurrences of GBS (Pollard and Selby, 1978; Galvan *et al.*, 1983). Tremor, which is quite uncommon in axonal polyneuropathies, has also been described in the demyelinating form of hereditary motor and sensory neuropathy (type 1) and in the demyelinating neuropathy associated with IgM paraproteinaemia (Thomas, 1984). The mechanism of this tremor and its relationship to demyelination and remyelination remains unclear.

From a review of the literature, it is difficult to ascertain the clinical characteristics of CRP because results have usually been pooled with the chronic progressive form of CIDP (Dyck *et al.*, 1975; Dalakas and Engel, 1981; McCombe *et al.*, 1987). However, analysis of the detailed report by Prineas and McCleod in 1976 allows the identification of 19 patients with CRP defined as two or more episodes each with an onset phase of more than 3 wks following which both symptoms and signs improved. Therefore there is some overlap in time-course with our patients. Only 15 of approximately 54 episodes (28%) were associated with an antecedent illness, mostly infection. It is possible, although unlikely, that the much higher incidence of potential triggering events in our patients (78%) represents ascertainment bias related to the more acute onset of symptoms, whereby patients are more likely to recall a recent infectious episode.

In Prineas and McCleod's series, five patients (26%) had facial weakness, one (5%) had ophthalmoparesis and two (11%) required ventilatory assistance during their most severe attack; these percentages would likely be much lower if computed as a function of the total number of episodes, as was done in the present study. Neurological examination was normal between relapses in three of their patients (subjects 1, 3, 7), who were among those with the shortest intervals from symptom onset to peak deficit. It is possible that the condition in these three patients corresponds to RGBS, as defined by our clinical criteria, rather than to CRP. It is noteworthy that many of their subjects showed a rapid apparent response to treatment with prednisone or with adrenocorticotropic hormone. Although the present study was not designed to assess properly the efficacy of corticotherapy in RGBS, no significant response was noted in our patients.

Results of CSF studies in our patients during recurrences and asymptomatic intervals are those expected in classical monophasic GBS (Asbury and Cornblath, 1990). Early during a recurrence and following recovery, CSF protein levels are normal or minimally elevated but increase by 1 wk after the onset of an attack. Serial measurements of CSF protein levels in CRP are reported in two studies (Prineas and McCleod, 1976; Dalakas and Engel, 1981). In both series, it is mentioned that CSF protein levels tend to remain elevated during remissions, albeit at levels below those during exacerbations. These observations suggest quiescence or resolution of the inflammatory response between episodes in RGBS and ongoing, though subclinical, inflammatory activity during remissions in CRP.

Results of nerve conduction studies in our patients are consistent with those reported in the literature for acute monophasic GBS (Brown and Feasby, 1984; Albers, 1987). Slowing of conduction velocities, prolonged distal latencies, conduction block or dispersion, which represent the cardinal features of acquired demyelinating polyneuropathies, were seen in most patients. Evidence of axonal degeneration in the form of fibrillation potentials and positive sharp waves, observed in some of our patients, has frequently been reported in GBS (Brown and Feasby, 1984; Albers, 1987). It is difficult to compare electrophysiological findings between RGBS and CRP since, to our knowledge, no systematic longitudinal studies have been performed in either case. In our two patients in whom nerve conduction studies were obtained both during the acute phase and during the asymptomatic interval, no significant improvement in conduction velocities or distal latencies was noted. This does not necessarily indicate ongoing disease activity during asymptomatic intervals in RGBS since nerve conduction abnormalities may persist following full functional recovery from acute monophasic GBS (Albers, 1987). It is therefore unlikely that nerve conduction studies will provide a strong basis to distinguish RGBS from CRP.

The nerve biopsy in patient 1, obtained in the acute phase of the fifth episode, revealed segmental demyelination, onion bulb formations and substantial endoneurial infiltration by inflammatory cells. These findings are relatively non-specific and indicate repeated episodes of demyelination and remyelination. Similar morphological observations have been made in CIDP (Dyck *et al.*, 1975), CRP (Prineas and McCleod, 1976) and in another case of RGBS (Pollard and Selby, 1978). Biopsies of SPN and DPN from patient 2, although performed during the third episode, showed no evidence of chronic disease. The predominant axonal changes may be explained by a focus or foci of inflammation and demyelination proximal to the biopsy site with secondary axonal degeneration. This explanation is strongly supported by the prior demonstration of a much delayed common peroneal F-response (72.7 ms) despite normal conduction velocity in the foreleg indicating demyelination proximal to the knee.

In conclusion, RGBS consists of multiple episodes of typical acute GBS, each bout varying considerably and unpredictably in severity and in the distribution of involvement with the possible exception of features of the Miller Fisher variant which seem more constant from one episode to the next. Tremor and enlarged nerves, unusual in GBS, followed multiple attacks. The CSF findings and EMG abnormalities during each individual episode are similar to those described in acute monophasic GBS. Nerve conduction abnormalities sometimes persisted between episodes. Nerve biopsy revealed onion bulb formations, indicating repeated demyelination and remyelination. Whether RGBS and CRP represent different conditions remains unclear. The distinction is blurred by the fact that the time course of the onset phase of individual attacks varies considerably in few patients (Prineas, 1971; McCombe *et al.*, 1987). Nevertheless, our observations on RGBS suggest that the rapid onset of symptoms with subsequent complete or near complete recovery, the high incidence of an antecedent illness, the apparently poor response to immunosuppressive therapy and the normal CSF protein levels at the onset of recurrences may be sufficiently distinctive from CRP to justify their separation until more definite evidence proves differently.

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TORSIONAL NYSTAGMUS

A NEURO-OTOLOGICAL AND MRI STUDY OF THIRTY-FIVE CASES

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SUMMARY

Thirty-five patients with torsional nystagmus (TN) underwent vestibular and ocular motor assessment and magnetic resonance image (MRI) scanning of the head. Patients were divided into two groups according to whether TN was predominant and present in primary gaze (Group I, 23 patients) or elicited by head positioning or gaze deviation and less prominent than other concurrent nystagmus (Group II, 12 patients). The main aetiologies in both groups were demyelination, vascular disease and posterior fossa tumours. In Group I, a frequent pattern of findings, occurring in 30–50% of cases, was a caloric canal paresis contralateral to the direction of the fast phases ('beat') of the TN, whereas the duration of horizontal caloric/rotational nystagmus and the slow-phase eye velocity of pursuit and of optokinetic nystagmus were all reduced in the direction of beating. The TN was more frequently and consistently modulated by vertical canal stimuli (head oscillation in roll) than by otolith stimuli (static tilt). Statistical analysis of the MRI showed significant overlap of abnormal MRI signals in the area of the vestibular nuclei, on the side opposite to the beat direction of TN. These results suggest that TN originates in a central imbalance of vertical semicircular canal function, resulting from lesions involving the vestibular nuclei on the opposite side of the TN. Group II was heterogeneous with no consistent pattern of neuro-otological findings, although lesions ipsilateral to the TN were frequent occurrence; in these cases cerebellar system lesions may have produced ipsilateral vestibular nuclei disinhibition.

INTRODUCTION

Movements of the eye around the visual axis are called cyclorotational or torsional, and nystagmus occurring in this plane is described as 'rotatory' or, more correctly, torsional nystagmus (TN). Physiological TN is elicited by rotational movements of the head in the frontal (roll) plane. The quick component of the nystagmus beats in the direction of head motion, i.e. during right ear down to right shoulder movements a fine TN beating towards the right shoulder can be seen. The slow component of eye movement, arising from stimulation of the vertical semicircular canals (anterior and posterior) and probably the otolith organs, is the torsional vesibulo-ocular reflex (VOR) which aides visual fixation of earth-fixed objects during such head movements. From what is known about the general organization of the VOR (Wilson and Jones, 1979; *see* Barnes, 1980, for review; Carpenter, R. H. S., 1988), the vestibular nerves and nuclei, the reticular formation, cerebellar and internuclear pathways and the III and

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IV nuclei, participate in this process. Accordingly, pathological TN could arise from an imbalance of neural activity in peripheral or central vertical canal and/or otolith ocular pathways.

The significance of TN is not well understood and, to our knowledge, no results of a large series of patients have been published. The lack, until the recent advent of magnetic resonance imaging (MRI), of appropriate imaging procedures to identify the site of lesions and the difficulty in recording torsional ocular movements, have been significant limiting factors. Oculographic techniques for use in the clinical setting, infrared and electro-oculography (EOG), are insensitive to movements in the torsional plane and alternative procedures are either laborious and expensive image-based techniques or semi-invasive such as the scleral search eye coil method (Collewijn *et al.*, 1985). Whilst coil recordings of TN in four patients have recently been published, the influence of vestibular stimuli on the TN in those patients was not studied (Morrow and Sharpe, 1988; Noseworthy *et al.*, 1988).

Here we report the results of an MRI study of patients with TN, using a statistical technique for the analysis of MRIs, together with results of conventional vestibular and ocular motor testing procedures. Although torsional ocular recordings were not available during this study, we will describe the effects of roll-plane vestibular stimuli on TN as observed by direct clinical inspection.

MATERIALS AND METHODS

Clinical material

Patients seen between 1985 and 1990 with 'jerky' (saw-tooth) TN visible by the unaided eye, whether isolated or in combination with other nystagmus, were studied. Patients with typical benign positional paroxysmal nystagmus (Dix and Hallpike, 1952) or with TN elicited by sound (Tullio phenomenon; Dieterich *et al.*, 1989), which have well-established labyrinthine origins, and patients with pendular TN, i.e. without distinct fast and slow phases, were excluded. Three patients with TN during positional testing, in whom central pathology was suspected, were included.

The diseases provoking TN in our patients are listed in Table 1, the more common being demyelinating and vascular lesions, followed by posterior fossa tumours.

TABLE 1 AETIOLOGICAL DIAGNOSIS IN 35 CASES OF TN

	Group	
	I	*II*
n	23	12
Male/female	12/11	3/9
Age (mean and range) (yrs)	41 (22–60)	44 (18–75)
Diagnosis		
Multiple sclerosis	8	3
Vascular	7	3
Posterior fossa tumour	2	3
Post encephalitic	2	0
Syringomyelia	1	0
Sarcoidosis	0	1
Traumatic	1	0
Peripheral positional nystagmus	0	1
Unknown	2	1

Since other associated nystagmus were often present, the patients were divided into two groups according to whether the TN was a prominent or a secondary ocular motor feature. The former constituted Group I (23 cases), which included patients with predominant or isolated TN in primary gaze and those with a purely TN evoked by lateral gaze. Group II was less homogeneous and numerous (12 cases), including patients with positionally elicited TN, one patient with paroxysmal TN and one with TN appearing only with fixation of distant objects but not during conventional examination. Also included in this group were cases of combined torsional-horizontal nystagmus elicited by lateral gaze (gaze-evoked nystagmus with a torsional component) and combined torsional-horizontal nystagmus in primary gaze in which the horizontal nystagmus was predominant and in the opposite right-left direction to the TN. Group I consisted of 11 females and 12 males with a mean age of 41 yrs, range 22–62 yrs, Group II included nine females and three males with a mean age of 44 yrs, range 18–73 yrs. Group I constitutes a homogeneous population and the main thrust of this paper, whereas Group II is less uniform and will be divided into small subgroups.

Vestibular and ocular motor assessment

Clinical examination. Examination of eye movements included saccades, pursuit, optokinetic nystagmus responses to a small motorized drum, doll's head, convergence and the observation of nystagmus in primary gaze, on 30 deg eye deviation up, down, right and left and during the positional Nylen-Hallpike manoeuvre The direction of the TN is defined by the direction of beat of the superior part of the eye to the patient's right or left shoulder.

Caloric testing. Following otoscopy, bithermal caloric tests were carried out as described by Fitzgerald and Hallpike (1942) by direct observation and measurement of the duration of the nystagmus response during visual fixation. Abnormalities in the duration of the caloric responses were identified using the formulae:

$$\text{Canal paresis }(\%) = \frac{(\text{L cold}+\text{L hot})-(\text{R cold}+\text{R hot})}{\text{L cold}+\text{L hot}+\text{R cold}+\text{R hot}}\times 100$$

$$\text{Directional preponderance }(\%) = \frac{(\text{L cold}+\text{R hot})-(\text{R cold}+\text{L hot})}{\text{L cold}+\text{L hot}+\text{R cold}+\text{R hot}}\times 100$$

Our normal limits, in the presence of visual fixation, are 6% for canal paresis and 9% for directional preponderance.

Electro-oculographic assessment. Horizontal eye movements were recorded with bitemporal DC EOG. In the presence of ocular disconjugacy, separate eye recordings were obtained. The VOR was assessed by whole-body rotation to velocity steps of ±40 deg/s, delivered in the dark by a motorized revolving chair. Smooth pursuit was investigated with a sinusoidally moving laser target at a frequency of 0.2 Hz and amplitude of ± 16 deg (peak velocity 20 deg/s). Optokinetic nystagmus (OKN) was generated by constant velocity rotation (±40 deg/s) of a full-field surrounding drum.

Slow-phase eye velocity was measured manually from the eye position records obtained with an ink jet recorder. The time constant of decay of the slow-phase velocity of the vestibular nystagmus was determined, defined as the time taken by eye velocity to decay to a value of approximately 37% of the peak velocity generated immediately after the velocity step. The two right-beating responses (one from rightwards rotation and one from stopping leftwards rotation) and the two left-beating responses were averaged. Rightwards and leftwards peak velocity of smooth pursuit and OKN were measured. Slow phase asymmetry (SPA) was assessed with the formula,

$$\text{SPA }(\%) = \frac{\text{R response}-\text{L response}}{\text{R response}+\text{L response}}\times 100$$

where 'response' was either time constant of the decay of the rotational nystagmus or peak velocity for pursuit and OKN. Normal values in our laboratory, at the stimulus parameters described above, are: OKN velocity = 27.16 deg/s, SD = 2.89 deg/s; percentage asymmetry = 1.40 SD = 1.07%; smooth pursuit velocity = 16.80 deg/s, SD = 2.20 deg/s, percentage asymmetry = 2% SD = 1.05%; vestibular (rotational) nystagmus time constant = 13.93 s, SD = 5.10 s; percentage asymmetry = 10% SD = 3.80% (range 4–15%). Normal limits of asymmetry used were, for pursuit and OKN velocity, 4% (mean ± 2 SD) and, for vestibular time constant, 15% (range, as time constants were not normally distributed). Vestibulo-ocular reflex suppression was qualitatively assessed from the paper records by noting the distinct appearance of nystagmus, whilst the subject was sinusoidally oscillated (peak velocity 40 deg/s, frequency 0.2 Hz) fixating a chair-fixed target.

Vestibular modulation of the TN. (i) The influence of *static otolith* input on the nystagmus was assessed by having the patient lying on the right and left sides for periods in excess of 1 min so that dynamic effects would die down (Fig. 1A). (ii) Combined *dynamic otolith and vertical canal* effects were investigated by oscillating the head about the naso-occipital axis (roll) with the subject positioned upright. This was achieved both by passive lateral flexion of the neck by the examiner holding the patient's head firmly with his two hands (Fig. 1B) and by using a gymbal system which allowed whole body rotation with the head rigidly clamped to the device (Fig. 1C). (iii) *Vertical canal* stimulation was achieved by oscillation of the head in roll with the subject in the supine position on a rotating couch which pivoted about the naso-occipital axis (Fig. 1D). All these examinations were carried out under normal laboratory illumination and, therefore, optokinetic stimulation was unavoidable during head oscillation. Modulatory influences on the nystagmus were assessed by direct observation by two observers (A.M.B and M.A.G.) during wide opening of the eyelids under strong illumination. Videotape films of the eye movements, including the vestibular modulatory influences, were frequently obtained; their assessment, however, was not 'blind'.

Magnetic resonance imaging and image analysis

Each patient had standard T_1- and T_2-weighted MRI scans performed on a 0.5 Tesla, Picker superconducting system. An image matrix of 128×256 was routinely employed, giving pixel dimensions of approximately 2.4×1.2 mm. The most informative transverse brainstem image was at the level of the ponto-medullary junction. Here the vestibular nuclei can be localized to the most lateral aspect of the floor of the fourth ventricle (Fig. 5). Lesions occurring in caudal medullary cuts were not resolved well enough in relation to brainstem anatomy for statistical analysis. Most patients had spin echo TR/TE 2000/60 and inversion recovery TR/TI 1500/500 sequences; lesions appearing as areas of increased signal (white) and low signal (black), respectively. The scans were reported blindly (E.P.G.H. du B.), using both scanning sequences and information from adjacent transverse brain slices, to determine the presence of lesions. The chosen brain slices from each patient were enlarged and traced onto acetate sheets, including unequivocal areas of abnormal signal and the brainstem/ventricular contours. Ponto-medullary and mid-brain slices were subsequently superimposed onto anatomical templates (obtained from overlaying 10 scans) and identified lesions were coded and analysed by a statistical method described fully in a previous study (Bronstein *et al.*, 1990*a*). Briefly, this tests whether a given number of lesions overlapping a defined area of brainstem, in a group of patients who share a common clinical finding, have occurred by chance.

GROUP I RESULTS

Vestibular and oculomotor assessment

Caloric testing

Twenty patients underwent caloric testing, although in two the results were uninterpretable because the induced caloric nystagmus was not horizontal ('perverted nystagmus'). The results are summarized in Fig. 2. In the 18 cases in whom the duration was measurable, a canal paresis was present in nine (50%) on the opposite side to the direction of the TN, i.e. a right beating TN had less responsiveness to irrigation of the left ear. In five cases (28%) there was no canal paresis and in three cases (18%) there was canal paresis on the same side of the TN. A directional preponderance of caloric nystagmus was found in seven cases (39%) and this was always ipsilateral to the direction of the TN, i.e. a right beating TN was associated with longer duration caloric responses to the right; there were no cases with contralateral directional preponderance. One case had bilaterally absent caloric (and rotational) responses.

Electro-oculographic findings

Rotational tests. Rotational tests were performed in 20 patients. In seven cases (35%) there was a directional preponderance of the time constant, i.e. a longer duration response,

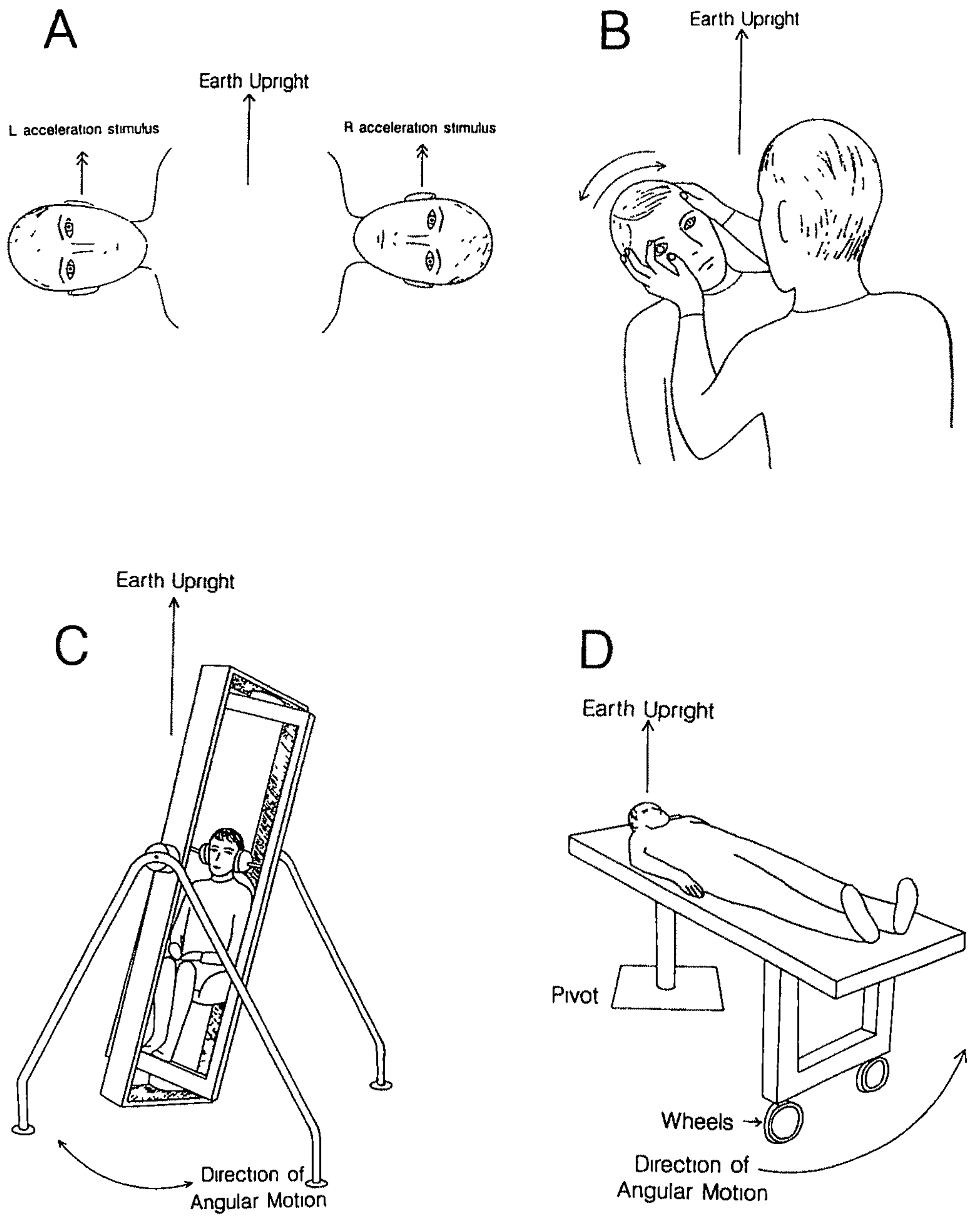

FIG. 1. Investigation of the effects of vestibular stimuli in roll (coronal plane) on TN. A, static head tilt investigates tonic otolithic influences. During sustained right ear-down tilt (left of Fig. 1A), 'g' force is applied along the inter-aural axis downwards and, therefore, this position is equivalent to a leftwards linear acceleration. B, dynamic roll motion about the upright. The examiner instructs the patient to relax the neck and to keep looking at the examiner's nose during passive oscillation of the head. This manoeuvre stimulates the vertical semicircular canals and the otolith organs. C, gymbals used to deliver controlled whole body oscillation in roll about the upright. D, turntable used to deliver controlled roll motion in the supine position. Oscillation in this position stimulates the vertical canals but, in contrast to B and C, the otolith organs maintain a fixed orientation with respect to gravity.

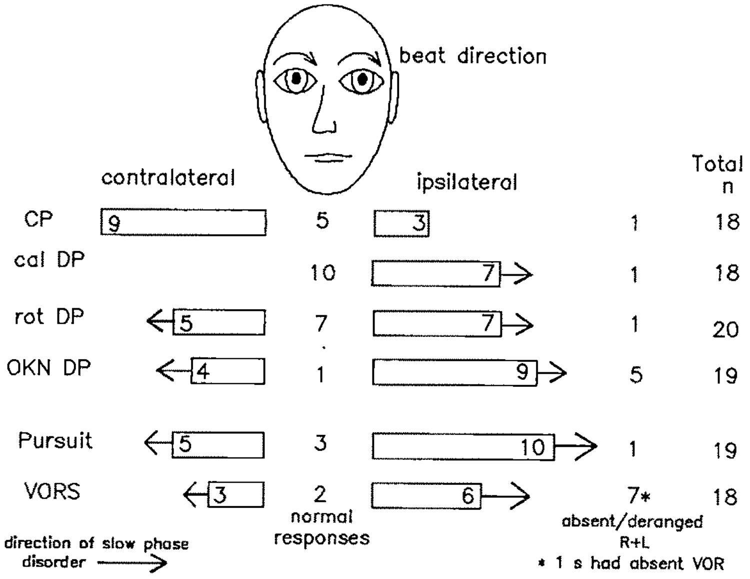

FIG. 2. Summary of vestibular and ocular motor investigations in the patients in Group I The numbers represent numbers of patients studied or patients with abnormal results in the same direction of the spontaneous nystagmus beats (ipsilateral) or in the opposite (contralateral). A sketch of a patient with left-beating TN is included. CP = canal paresis; cal = caloric; rot = rotational; DP = directional preponderance; VORS = vestibulo-ocular reflex suppression.

for the nystagmus beating ipsilaterally to the TN (Fig. 2). There was no significant asymmetry in seven cases (35%), a case with absent rotational responses (5%) and directional preponderance contralateral to the TN in five cases (25%). Vestibulo-ocular reflex suppression was reduced during head rotation in the direction of the TN in six patients (35%), bidirectionally in six (35%), in the opposite direction to the TN in three (18%) and normal in only two patients (12%).

Smooth pursuit and OKN. Pursuit was almost always abnormal in the patients with TN (Fig. 2). Ten cases (53%) had slow phase asymmetry with the greatest impairment of smooth eye movement velocity in the same direction as the nystagmus, five cases (26%) contralaterally to the nystagmus and symmetrical in four patients (21%).

Optokinetic nystagmus was usually abnormal, following a similar laterality pattern to that of pursuit and VOR suppression (Fig. 2). Nine patients (47%) had abnormal slow phase asymmetry with the slower velocity in the same direction of the TN, four patients (21%) had asymmetrical OKN with the slower eye velocity contralateral to the nystagmus and six patients (32%) had symmetrical OKN.

Vestibular modulation of the TN

Static tilt and dynamic roll motion of the head

The effect of static tilt was investigated in 22 of the 23 patients but it did not produce consistent, lateralized effects on the TN (Fig. 3). Tilt in the direction of the nystagmus, i.e. right-beating TN investigated in the right ear down position, increased nystagmus

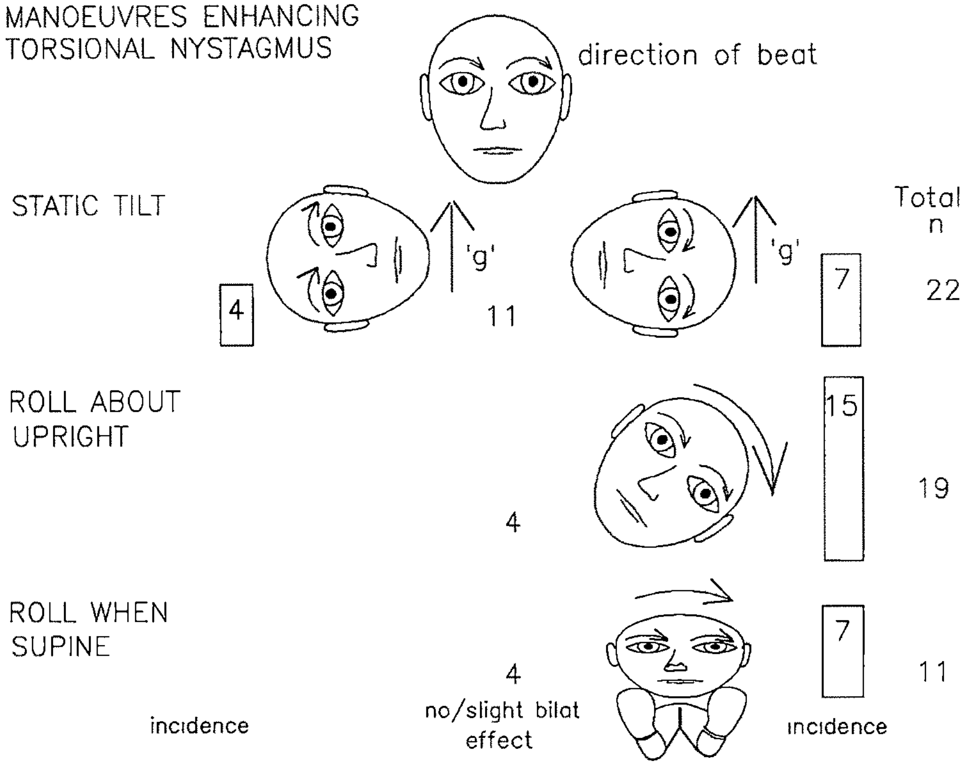

FIG. 3. Modulation of the TN by roll vestibular stimuli in Group I. Figures are number of patients. Results are presented as manoeuvres enhancing a spontaneous left beating TN Angular head motion in the beat-direction of the TN enhanced the nystagmus in the majority of patients.

activity in seven patients (32%). This would be the expected effect of gravitational accelerati on ('g') on the nystagmus in that rightwards tilt induces, in normal subjects, sustained counter-torsion of the eyes in the opposite direction (Diamond and Markham, 1983). However, in four patients (18%), tilt contralateral to the nystagmus had an enhancing effect, whereas in 11 patients (50%) the nystagmus was either not changed or slightly enhanced by static tilt in either direction.

During roll motion of the head about earth upright (investigated in 19 patients) ocular counter-rolling was generally asymmetrical, with more active nystagmus induced during head motion in the same direction of the nystagmus, i.e. right-beating TN was increased during movement of the head towards the right shoulder. This was seen in 15 cases (79%) and would be the normally expected enhancement by combined vertical canals and dynamic otolith mediated activity. In three cases (16%) ocular counter-rolling appeared symmetrical and in the case with absent caloric and rotational responses, ocular counter-rolling was absent, the spontaneous TN persisting unchanged through head motion.

In 11 patients ocular counter-rolling was also elicited in the supine position by means of the rotating couch depicted in Fig. 1D. In seven patients (64%) there was the expected canal mediated enhancement with more active TN during head motion in the same direction of the spontaneous TN and in four patients (36%) the nystagmus response appeared symmetrical.

There were two patients in whom ocular counter-rolling in the upright position was asymmetrical, i.e. enhanced in the ipsilateral direction of the TN, but symmetrical during investigation in the supine position. Such effect could be expected from a dynamic otolith input arising during head reorientation with respect to the gravity vector, as depicted in Fig. 1. However, there may be a dissociation between static and dynamic otolithic effects (Barratt *et al.*, 1987), for in neither of these patients was the dynamic enhancement in the same direction as that produced by static tilt. In one of the patients, static tilt to either side had a small enhancing effect on the spontaneous TN (strikingly, the TN was strongly affected by sagittal position since it reversed direction during supine and increased whilst prone). In the other patient, static tilt had an enhancing effect on the TN which was opposite to that induced dynamically.

Skew eye deviations

The presence of skew deviation [i.e. vertical ocular divergence caused by a disturbance of supranuclear (or prenuclear) innervation of vertically acting motor neurons] is relevant to TN because common mechanisms may be involved in their origin. Due to the small numbers, patients with skew deviation from Group I (seven patients) and Group II (two patients) will be considered together.

Conceivably, a right/left skew (i.e. the right eye is higher than the left) occurs because the eyes rotate in an orbital trajectory around the nasum-occipital axis, in this example in the clockwise direction as viewed by the observer (Fig. 4). In eight out of the nine cases the direction of the slow phase of the TN was in the same direction as that of the skew deviation (i.e. a right-beating TN is associated with a right/left skew deviation) (Table 2), suggestive that the skew and TN arise from impairment of related mechanisms.

In our patients with skew deviation, the TN was enhanced by dynamic head roll motion in the direction of the TN and inhibited by contralateral motion, in four out of seven

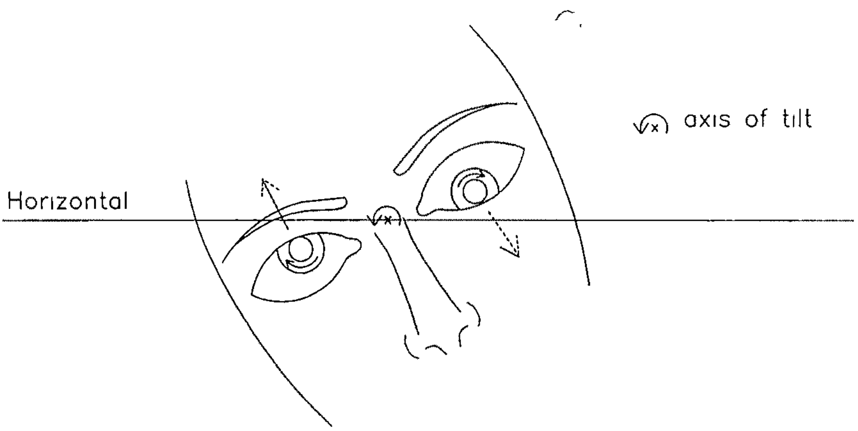

FIG. 4. Compensatory eye movements in response to head tilt to the right about the naso-occipital axis. Arrows indicate the direction of theoretical slow-phase compensation. The torsional movement is well documented but a normal 'vestigial' skew has not been demonstrated in man

TABLE 2 SUMMARY OF FINDINGS IN NINE PATIENTS WITH TN AND SKEW DEVIATION

Patient	Nystagmus direction	*TN enhancement*		Skew**	MRI
		*Tilt**	*Motion**		
Group I					
1	L	Bilat	Ipsilat	R↑ L↓ (quick)	R mid-brain
2	R	Bilat	Ipsilat.	R↑ L↓ (slow)	L mid-brain
3	L	Nil bilat.	Symmetric counter-rolling	L↑ R↓ (slow)	R ponto-med
4	R	Contralat	Ipsilat.	R↑ L↓ (slow)	R+L pons
5	L	Contralat.	Not done	L↑ R↓ (slow)	No MRI
6	L	Slight ipsilat	Symmetric counter-rolling	L↑ R↓ (slow)	No lesion on MRI
7	L	Slight bilat.	No response (absent vestibular function)	L↑ R↓ (slow)	L cerebellar (computerized tomography scan)
Group II					
8	R	Slight bilat	Not done	R↑ L↓ (slow)	R Pons./mid-brain
9	L	Ipsilat	Ipsilat	L↑ R↓ (slow)	R+L pons

*Torsional nystagmus enhancement by roll tilt/motion Ipsilateral (ipsilat.): the TN is enhanced by static head tilt or dynamic head motion towards the same side of the beat direction of the spontaneous TN. Controlateral (contralat.): TN enhancement by tilt/motion to the opposite side of the beat direction. Bilateral (bilat.): TN enhanced by tilt/motion to both right and left

**The hyperdeviated eye is given first. The congruency of the skew with the TN is given in brackets: 'slow' means that the vertical ocular divergence is congruent with the slow phase of the TN, 'quick' means that the skew occurred in the direction of the quick phase of the TN

Ponto-med. = ponto-medullary junction.

tested; it was unchanged in the patient with absent vestibular function and symmetrical in two.

The effect of static tilt on the TN, investigated in all patients with skew, was variable. The spontaneous TN was enhanced with both right and left ear down in three patients, enhanced by tilt in the direction of beating in two, enhanced by tilt away from the beat in two; there was a small bilaterally tilt-induced suppression in one and no effect in another patient. In the latter patient, with a left/right skew, the magnitude of the skew and diplopia increased with tilt to the left, i.e. in the expected direction from gravitational acceleration. The effects of dynamic stimulation on the skew deviation itself could not be assessed reliably in any of the subjects. In general, therefore, dynamic stimuli (otolith/vertical canal) rather than static-otolith activity (tilt) seems to have a stronger influence on the nystagmus seen in patients with TN-skew deviation.

Magnetic resonance imaging findings and image analysis

Because of relevant vestibular structures present in the cuts neighbouring that of the emergence of the vestibulo-acoustic nerve (ponto-medullary junction), images from a cut immediately caudal or rostral were included for the statistical analysis of the MRIs. This caused an enlargement of the region analysed to a total of 15 mm in the caudal-rostral axis. A one by one analysis of different cuts would not have produced enough data to process the images collectively, hampering the statistical investigation. Some of the structures contained in this area, including the vestibular nuclear complex projected onto a two-dimensional sketch, are shown in Fig. 5.

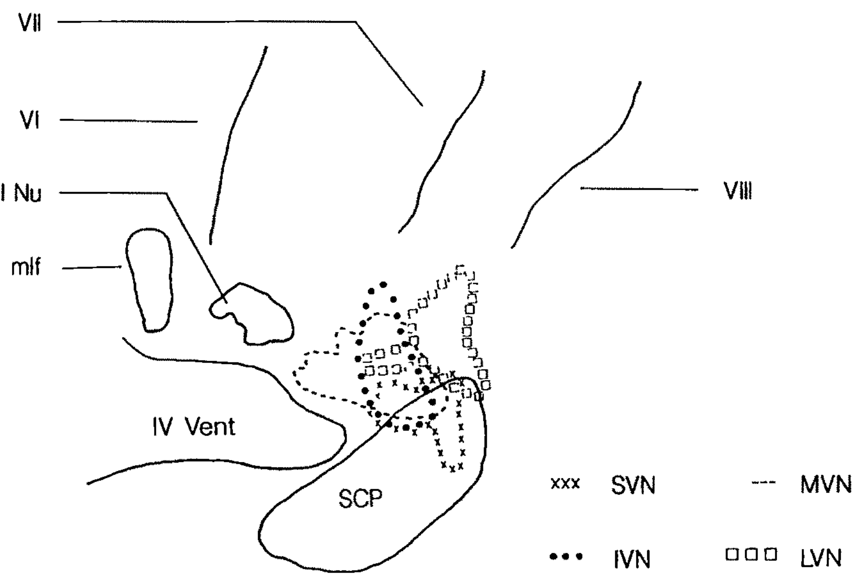

FIG 5. Neural structures present at the ponto-medullary junction The right side only is shown, in a similar position as the diagram in Fig. 7. VI, VII and VIII = intra-brainstem fasciculi of these cranial nerves, Vi Nu = abducens nucleus, mlf = medial longitudinal fasciculus, SCP = superior cerebellar peduncle. Graphic symbols depict the superior (S), medial (M), inferior (I) and lateral (L) vestibular nuclei (VN). The apparent overlap between the various VN and the SCP resulted from presenting the total length of the VN complex on a two-dimensional sketch, taking into account the volume effect in the MRI cuts used.

Eighteen patients had MRI scans. In three cases the MRI of the posterior fossa were normal. Of the 15 cases with brainstem lesions, 11 had abnormal images in the ponto-medullary region. Of these, six patients had bilateral lesions, five had lesions contralateral to the TN, i.e. a right-beating nystagmus showed lesions in the left ponto-medullary region, and no case had isolated lesions ipsilateral to the TN. Individual representative scans can be seen in Fig. 6. The result of the statistical analysis of the images is presented in Fig. 7, which shows that there was significant ($P < 0.05$) overlapping in boxes P10, P11 and O11. In the more lateral boxes (P10 and P11) the main structures present are the medial, inferior and superior vestibular nuclei; a small portion of the lateral vestibular nucleus and of the superior cerebellar peduncle are also contained in that area. In the more medial box (O11) the main structures are part of the abducens and medial vestibular nuclei.

Six out of the 15 patients had mid-brain lesions but since in three of these there were also lesions in the ponto-medullary junction, the significance of the mid-brain lesions is equivocal. The lesions in the three patients with isolated mid-brain lesions were contralateral to the TN in two and ipsilateral in one. If all six patients are considered, the side of the lesions was contralateral to the TN in three, bilateral in two and ipsilateral in one. Statistical analysis of the MRIs of this region did not show areas with significant overlap.

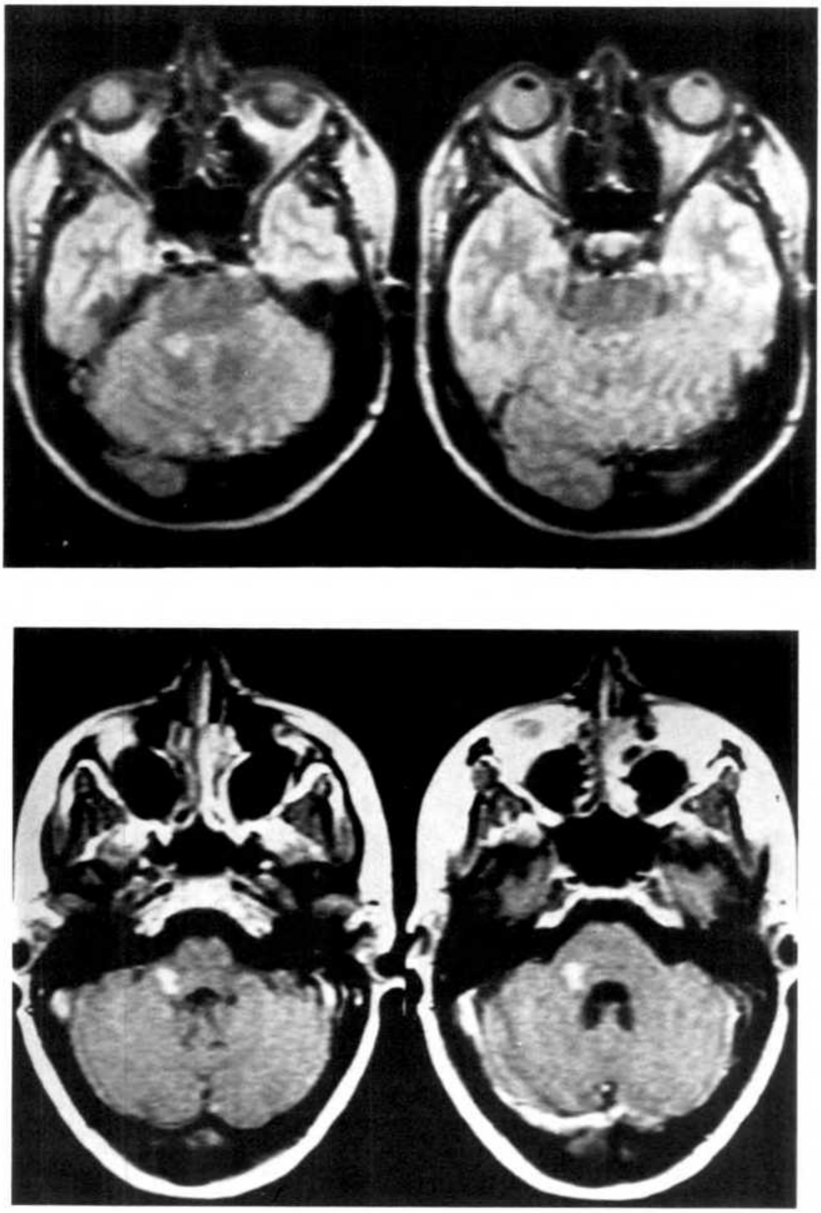

FIG. 6. Representative scans of two patients with left-sided ponto-medullary junction lesions on MRI and right-beating TN.

GROUP II RESULTS

As already mentioned, this group is non-uniform and therefore a combined clinical-MRI summary of the data is presented for each subgroup. Caloric and EOG data did not reveal any consistent pattern.

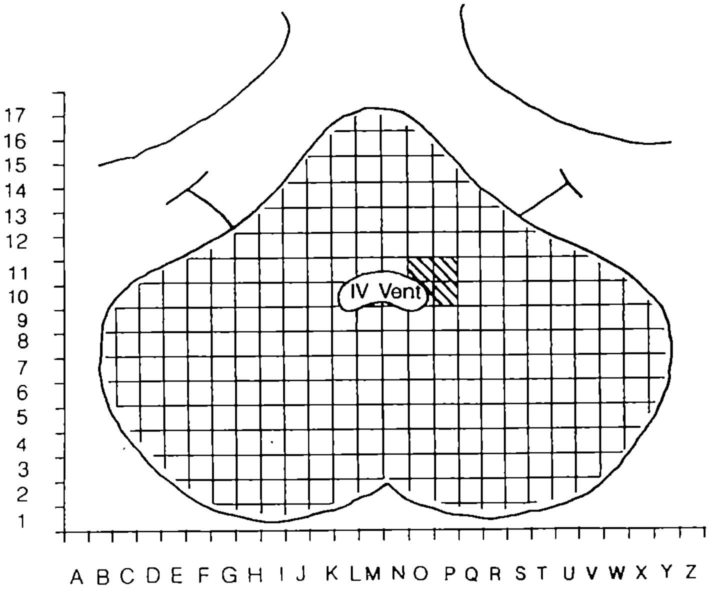

FIG. 7. Diagram showing statistically significant ($P < 0.05$) overlap of abnormal MRI signal in the TN patients in Group I. The neural structures contained in this region, lateral and anterolateral to the IV ventricle, can be found in Fig. 5.

'Atypical' positional nystagmus

Three patients with TN appearing only during the Nylen-Hallpike manoeuvre were studied because the nystagmus did not follow the pattern of benign paroxysmal positional vertigo (Dix and Hallpike, 1952) and/or because of additional neurological signs. In two patients a causal lesion was found on imaging (an ependymoma and an infarct) at the ponto-medullary level, on the side of the TN beat direction. In the third case no cause was found and a peripheral vestibular cause was presumed.

Gaze evoked horizontal nystagmus with a 'torsional component'

One of the three patients studied had demyelinating disease but no relevant posterior fossa lesion identified by MRI. One patient had a vascular pontine lesion and the other a ponto-mesencephalic arterial-venous malformation, both on the same side as the nystagmus.

Horizontal versus torsional nystagmus

In four cases a horizontal nystagmus was present combined with a TN which was of less magnitude and in the opposite right/left direction to the horizontal. Two patients had multiple sclerosis (in one case combined with a pineal mass) and the others a subarachnoid haemorrhage due to a ruptured PICA-vertebral artery aneurysm and neurosarcoidosis. No patterns of laterality with respect to the nystagmus could be found in these patients. A similar pattern of opposing direction torsional/horizontal nystagmus has been produced in monkeys by lesions in the vestibular nuclei (Uemura and Cohen, 1973).

Miscellaneous

One patient had 'paroxysmal' TN but, in between episodes, his neurological, vestibular and oculo-motor examination was normal; the MRI showed bilateral pontine abnormalities. It was thought that the picture might be compatible with vascular or demyelinating disease. One patient with a recurrent posterior fossa glioma and left-sided ponto-medullary abnormality on MRI had, in addition to various oculo-motor signs, the unusual appearance of left-beating low amplitude TN when she fixated objects several metres away but not during fixation of nearby objects or during conventional examination.

DISCUSSION

The main points for discussion emerging from the data relate to (i) the site of lesions, central or peripheral, giving rise to TN; (ii) the functional nature of the TN, as derived from vestibular and oculo-motor testing; (iii) the influence of roll stimuli, canalicular or otolithic, on TN and its relationship to skew eye deviations; (iv) MRI in topographical diagnosis. The discussion will follow this order and, unless specifically mentioned, will be focused on Group I.

Torsional nystagmus, central or peripheral vestibular sign?

None of our patients had spontaneous TN due to a peripheral vestibular lesion. This may be somewhat surprising since paroxysmal peripheral vestibular disorders, such as benign positional vertigo or Tullio phenomenon, both excluded from this study, generate strong TN. However, unilateral denervation of the posterior canal alone does not provoke TN, as shown by cases undergoing this surgical procedure for the treatment of benign paroxysmal positional vertigo (Gacek, 1984). It is possible that vestibular tonus in the roll plane is much more tightly controlled than that for the horizontal plane, the former using information from two canals (anterior and posterior) and the otolith organs from each side, whereas the latter basically relies on one horizontal canal per side. Accordingly, a selective anterior or posterior canal lesion might not upset vestibular tonus in roll sufficiently to generate nystagmus.

Although unilateral involvement of both vertical canals, with or without the utricle, could give rise to TN, such a selective lesion would be extremely rare. Usually, peripheral labyrinthine disease involves the horizontal as well as the vertical canals, resulting in a combined horizontal-torsional nystagmus, rapidly suppressed by central compensatory mechanisms. In clinical practice, a TN should be considered of central origin, although it may be theoretically possible to observe a peripheral TN during the acute phase of a highly selective labyrinthine lesion. Similarly, a practical conclusion can also be drawn from the three cases described in Group II with positional TN not typical of benign positional paroxysmal vertigo (Dix and Hallpike, 1952). The well-established risk of a central disorder in such cases was reflected in our material as two of these had central lesions.

Neuro-otology of TN

Vestibular and eye movement investigations in patients with TN revealed a high incidence of central types of abnormalities. These data, with exceptions, show some laterality trends; caloric canal pareses were more frequent on the side opposite the TN, whereas caloric (directional preponderance), rotational, optokinetic, pursuit and vestibulo-ocular reflex suppression examination revealed abnormalities of slow-phase eye movement predominantly in the beat direction of the TN.

In a recent study, using the same techniques in patients with caloric canal pareses of central origin, we found that the statistically significant MRI lesions were in the area of the vestibular nuclei on the same side of the canal paresis (Francis *et al.*, 1992); this was contrary to our expectations, as the scarce experimental evidence points to the vestibular nerve root-entry zone as the site responsible for central canal paresis (Uemura and Cohen, 1973). In the light of our previous results, therefore, the findings reported here are compatible with a lesion in the area of the vestibular nuclei, causing ipsilateral canal paresis and contralateral TN. Although caloric testing is restricted to the horizontal plane, the findings would seem to apply to torsional eye movements and suggest a similar organization in these two planes.

Otolith or vertical canals?

The interaction between vestibular stimuli and TN was investigated clinically by observing dynamic ocular counter-rolling, mainly dependent on canal activity, and by the effects of static tilt on the spontaneous TN. An obvious disadvantage of this study is the lack of quantitative analysis of the TN due to well-known technical difficulties for recording torsional eye movements in the clinical setting, particularly if complex roll stimuli are to be delivered. Clinical inspection of the TN may have overlooked small effects but similar observations in experimental animals have been valuable (Uemura and Cohen, 1973).

In our patients, the TN was more systematically modulated by dynamic head-roll motion than by static tilt. In about 70% of cases the TN enhanced in the direction of head motion and diminished in the opposite direction, whereas no clear pattern was found for static tilt. Such responses suggest that TN occurs in the main as a result of an imbalance of semicircular canal activity between the two sides of the brainstem and that, although static otolith activity may play some modulatory effect, this seems to be less specific and weaker than canal-related activity. The situation is somewhat similar to downbeat nystagmus, in which the imbalance primarily responsible for the nystagmus is often of canal origin (Baloh and Spooner, 1981), frequently modulated or provoked by certain head positions (Gresty *et al.*, 1986).

The association between TN and the presence of a skew eye deviation is also of significance, as it has been proposed that skew deviations result from a tonic imbalance of otolith signals (Keane, 1975; Halmagyi *et al.*, 1979, 1990; Brandt and Dieterich, 1987). In our patients, the skew eye deviation usually was in the same direction as the slow phase of the TN, which suggests that these two phenomena either have the same origin or are produced by disruption of closely related mechanisms. One should therefore ask, what normal function, when disrupted, could give rise to TN and skew eye deviation? In animals with laterally placed eyes, the stability of visual images during head-motion roll is preserved by the torsional VOR together with a skew deviation of the eyes, so that the eyes remain aligned with earth horizontal (Simpson and Graf, 1981). In man, however, it is not known whether a vestigial skew response to head-roll motion/tilt exists and only the dynamic torsional component is well developed (Fig. 4) (Collewijn *et al.*, 1985). Furthermore, to our knowledge, no reports are available showing that pathological skews are sensitive to linear head acceleration or tilt, as it would be expected if skew deviations resulted from lesions involving otolith-ocular pathways. In our patients, even though no assessment of the skew was possible during roll motion, at least the TN was more clearly modulated by dynamic than by static stimuli. Therefore, if skew deviations and TN share common mechanisms it is possible that an asymmetry in vertical canal activity underlies the vertical disconjugacy of the eyes.

The synaptic connectivity of the canals would suggest that they can cause a skew as well as TN. By way of example, rightwards head movement in roll activates both the right anterior canal, which in turns excites the ipsilateral superior rectus and the contralateral inferior oblique, and the right posterior canal, in turn activating the ipsilateral superior oblique and the contralateral inferior rectus (Cohen and Suzuki, 1963; Suzuki *et al.*, 1964; Highstein, 1971; Baker *et al.*, 1973). Although the main effect of such activity would be to generate eye torsion, the prevalent vertical vector of both recti (right superior and left inferior) might induce a skew deviation and, accordingly, lesions could result in combined TN/skew deviation. However, it is most likely that a lesion would interfere both with canal and otolith activity as it is known that considerable convergence exists between these pathways (*see* Wilson and Jones, 1979, for review). In this context, it is usually assumed that the more 'tonic' skew deviation relates to otolith mechanisms, whereas the TN relates to more 'dynamic' canal mechanisms. Although this is an attractive hypothesis, skew deviations might be canal-related and the lack of 'skewing' nystagmus might be simply due to the impossibility of generating vertical fast phases in opposite directions in the two eyes (Vilis *et al.*, 1989); the slow phase deviation might not be corrected by nystagmus beats as with canal ocular reflexes in other planes. A more precise knowledge of these mechanisms will require binocular tridimensional recordings of eye movements during head-roll motion. We must conclude that the question is still open as to whether a lesion of vertical canal pathways can give rise to skew ocular deviations.

Magnetic resonance imaging in topographical diagnosis of TN

Analysis of MRIs was an important aspect of this study as the location of lesions responsible for TN is not entirely clear. It is known that the nystagmus present in cases of Wallenberg's syndrome is frequently torsional and thought to arise from vestibular nuclear damage (Baloh *et al.*, 1981; Estañol and Lopez-Rios, 1982; Morrow and Sharpe, 1988; Waespe and Wichmann, 1990). In an attempt to minimize the difficulties posed by the presence of multiple areas of abnormal MRI signal, lack of direct visualization of brainstem nuclei and possible variations in head positioning, we have statistically analysed the MRIs with a technique previously validated in cases where the anatomical basis of a certain clinical sign was predicted from anatomical or animal studies (Bronstein *et al.*, 1990*a*,*b*).

Preliminary assessment of the individual MRI scans in the patients in Group I showed a clear prevalence of lesions in the ponto-medullary region, either bilaterally or on the opposite side of the TN. Lesions in the uppermost pons and mid-brain were less frequent and no pattern of laterality could be established. This trend was reflected during statistical analysis of the data in that the only areas of significant overlap were at the ponto-medullary level, contralaterally to the TN beat.

The areas showing statistically significant overlap include, in the main, the superior

(S), medial (M) and inferior (I) vestibular nuclei. This is in agreement with the findings of lesions of the vestibular nuclei in monkeys which frequently resulted in contralaterally directed TN, often combined with horizontal or upwards vectors added (Uemura and Cohen, 1973), and with the fact that the S, M and I vestibular nuclei send direct projections to III and IV nucleus motor neurons controlling ocular torsion (Cohen and Suzuki, 1963; Suzuki *et al.*, 1964; Highstein, 1971; Baker *et al.*, 1973; Carleton and Carpenter, 1983; Carpenter and Cowie, 1985; Carpenter M. B., 1988). These monosynaptic pathways travel in the medial longitudinal fasciculus and the superior cerebellar peduncle, but it is not clearly established that, if lesioned, TN would develop. Furthermore, vestibular nystagmus, torsional or otherwise, is not a feature of isolated medial longitudinal fasciculus lesions. Thus, although vestibular nuclear lesions as seen in our patients could provoke TN by disrupting the site of origin of mono-synaptic pathways, effects derived from disruption of less well-known, polysynaptic vestibulo-ocular pathways are also likely (Ezure and Graf, 1984; Lisberger, 1984).

A feature in Group I was the absence of statistically significant overlap of abnormal MRI signal in the cerebellum or the mid-brain. We had initially speculated that lesions of the cerebellum might produce ipsilateral disinhibition of the vestibular nuclei (Yamamoto, 1979), in turn leading to ipsilaterally beating TN. In fact, in certain patients with 'gaze evoked horizontal-torsional nystagmus' and 'positionally elicited' TN (Group II) and two patients in Group I (one with absent vestibular function plus a cerebellar lesion on the beat side of the TN), ipsilateral cerebellar lesions were almost certainly responsible for the TN. So it would seem that in certain circumstances, for instance unusual otolith input (positional manoeuvre), concurrent gaze evoked cerebellar nystagmus or lack of peripheral vestibular input, a cerebellar lesion may have more impact on central vestibulo-ocular control and the 'disinhibition' mechanism proposed above could generate TN.

It was thought that the mid-brain could also be a region involved in cases of TN because skew deviations are known to arise with lesions in this area (Halmagyi *et al.*, 1990; Lueck *et al.*, 1991), probably by involving the interstitial nucleus of Cajal. In Group I, however, there were only three cases in whom the TN could have been reasonably attributed to mid-brain lesions but, since the TN was contralateral to the lesions in two and ipsilateral in one, no clear pattern of laterality emerges. It can be concluded that, although TN may arise from mid-brain lesions, this seems to be comparatively rare and of uncertain lateralizing value.

Conclusions

In the group of patients with predominant or purely TN (Group I), vascular and demyelinating brainstem disease were the most frequent aetiologies. Common findings were a canal paresis during caloric testing, contralateral to the TN, longer duration of horizontal caloric and rotational nystagmus in the same direction of TN and a reduction of slow phase eye velocity of pursuit and full-field optokinetic eye movements ipsilateral to the TN. The TN was more frequently and consistently modulated by vertical canal (head oscillation in roll) than by otolith (static tilt) stimuli. Statistical analysis of the MRIs showed significant overlap of abnormal MRI signal in the area of the vestibular nuclear complex on the side opposite to the TN. These results suggest that TN is a central nystagmus which originates in an imbalance of vertical semicircular canal function resulting from lesions involving the vestibular nuclei on the contralateral side of the TN.

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ENDURING DYSMETRIA AND IMPAIRED GAIN ADAPTIVITY OF SACCADIC EYE MOVEMENTS IN WALLENBERG'S LATERAL MEDULLARY SYNDROME

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SUMMARY

Saccadic eye movements and the adaptive control of their amplitudes were examined in patients with Wallenberg's lateral medullary syndrome. Half of the patients had permanent saccadic dysmetria. Their primary saccades had asymmetric amplitudes: those made in response to an ipsilateral target step (i.e. to the lesion side) tended to be hypermetric and saccades made in response to a contralateral target step were strongly hypometric. Multiple correction saccades were needed for target fixation.

The adjustment of the amplitude of artificially induced hypermetric saccades, called gain adaptivity, was examined experimentally by using double target steps. The first target step elicited the primary saccade which triggered a further target displacement. This second, intra-saccadic target displacement was opposite to the first target step and caused the primary saccade to overshoot the final target position. In this way a post-saccadic target position error was generated which had to be corrected for foveal fixation. With repetition of this stimulus sequence the saccadic control system of normal subjects made an adjustment in amplitude of the main saccade such that the overshooting gradually diminished. After a few hundred trials primary saccades became orthometric with respect to the final target position; in respect to the first target step they were, however, strongly hypometric. The experimental data show that patients with Wallenberg's syndrome had a reduced capability to readjust saccadic amplitude. This observation together with the enduring saccadic dysmetria suggest that adaptive gain control of saccades is impaired in patients with lesions restricted to the dorsolateral medulla. It is speculated that these lesions most likely disrupt olivo-cerebellar pathways which are believed to be of paramount importance in visuo-motor adaptation of the cerebellum.

INTRODUCTION

Rapid eye movements used to change the direction of sight are called saccades. A prominent feature of the saccadic system is the velocity and precision of saccades in attaining a target permitting good visual acuity. Saccades are so fast that the nervous system is unable to use visual feed-back to improve their execution. The saccadic system operates in a preprogrammed open-loop fashion and is therefore more sensitive to external and internal disturbances than a system which is controlled by a continuously operating feed-back loop. If errors in acquiring a target occur regularly and consistently, an adaptive mechanism is desirable which detects inappropriate saccadic performance and which recalibrates sensory input-motor output by incorporating error information in the motor program (Miles, 1983). Evidence for rapid and slow adaptive processes by which the

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brain maintains optimal oculomotor performance has been accumulating and the most important source of error signal to detect poor performance is visual experience (Henson, 1978; Optican, 1985; Optican and Miles, 1985; Deubel *et al.*, 1986; Optican *et al.*, 1986; for overview *see* Berthoz and Melvill Jones, 1985). Where and how the adaptive control or saccadic trajectory occurs is unknown but the cerebellum has been linked to the adaptive control or 'self-repair' of eye movement parameters (Ito, 1972; Robinson, 1975; Optican and Robinson, 1980).

In normal subjects, primary or main saccades slightly undershoot a fixation target; overshooting very rarely occurs. A correction saccade in the direction of the main saccade is made with a short latency suggesting preprogramming. It is crucial for the saccadic system to avoid overshooting of the target since the generation of a corrective saccade into the opposite direction is time-consuming due to the necessary inter-hemispheric transfer of the visual information about target position (Henson, 1978; Deubel *et al.*, 1986). With the assumption that visual error signals are essential for the adaptation process, adaptivity of saccadic amplitude was studied by inducing hypermetric saccades artificially (McLaughlin, 1967; Miller *et al.*, 1981; Deubel *et al.*, 1986; Deubel, 1989). Subjects had to track a target which moved in double steps. The first step elicited a primary saccade which triggered a further displacement of the target in the opposite direction of the initial target step. This intra-saccadic target displacement made the primary saccade too large (hypermetric) in respect to the final target position. Thus a consistent post-saccadic position error of the target was elicited which was eliminated by correction saccades. With repetition of the stimulus sequence the amplitude of the primary, hypermetric saccade was progressively reduced in normal subjects within minutes and after only a few hundred trials saccades became orthometric with respect to the final target position (Deubel *et al.*, 1986; Deubel, 1989). These changes in saccadic gain (size of primary saccade divided by the size of the initial target excentricity) result from the operation of a visually mediated rapid adaptive mechanism that normally functions to minimize post-saccadic position error and to prevent hypermetria of saccades. This experimental paradigm was applied to patients with the lateral medullary, retro-olivary or Wallenberg's syndrome which are known to have distinct saccadic eye movement disturbances. There have been numerous descriptions of deficits in voluntary and involuntary movements of the limbs and eyes in humans with posterior fossa lesions. In contrast, there has been a paucity of studies concerned with adaptive behaviour in these patients (Gauthier *et al.*, 1979; Weiner *et al.*, 1983; Zee and Optican, 1985; Sanes *et al.*, 1990). The general finding is that patients with cerebellar lesions have reduced visuo-motor adaptation. Examination of adaptivity in patients with Wallenberg's syndrome in the chronic stage of their disease is of special interest. On the one hand, these patients have a lesion outside the cerebellum in the dorsolateral aspect of the medulla oblongata. These lesions most probably interrupt olivo-cerebellar pathways. Damage to these pathways is assumed to be of major importance for the observed oculomotor disturbances (Hoyt and Frisén, 1975; Waespe and Wichmann, 1990). On the other hand, experimental evidence suggests a crucial role of olivo-cerebellar pathways originating in the inferior olive for the adaptive control of motor performance by the cerebellum (Ito and Miyashita, 1975; Llinás *et al.*, 1975; Ito and Kano, 1982; Ito *et al.*, 1982; McCormick *et al.*, 1985). The idea is that the climbing fibre inputs to the Purkinje cells provide 'teaching' signals and the mossy fibre inputs provide important contextual information to establish new associations or to change the strengths of connections based on the climbing fibre inputs to the Purkinje cells (Marr, 1969). These ideas and assumptions have generated considerable interest in testing visuo-motor adaptivity of saccades in Wallenberg's patients.

PATIENTS AND METHODS

This report is based on 13 patients with an ischaemia in the lateral medulla and/or the cerebellar territory of the posterior inferior cerebellar artery (PICA), and on two further patients with a sporadic cerebellar cortical atrophy. In most of these patients, eye movement abnormalities in the acute phase of the disease were marked and magnetic resonance imaging (MRI) studies allowed localization of the underlying pathology (Table 1). Patients were examined several months or years after onset of their illness in the chronic stage. Ten patients (patients 1 – 10) had infarction in the (dorso)lateral medulla (i.e. Wallenberg's syndrome) and four of these (patients 1 – 4) had further infarction in the cerebellar territory of the PICA.

Three further patients (patients 11 – 13) had infarction in the cerebellar territory of the PICA but none in the lateral medulla (Table 1). Twelve patients had MRIs; patient 6, who had a right-sided medullary lesion had a brain computerized tomography (CT) investigation which disclosed no cerebellar involvement. For patho-anatomical details we refer to our previous study (Waespe and Wichmann, 1990). Table 1 summarizes the diagnosis and the location and extent of the lesions. Mean age of the 13 patients with ischaemic infarction was 52 yrs (range 33 – 68 yrs; two females, 11 males). Five patients had also participated in our previous study on visual-vestibular interaction (Waespe and Wichmann, 1990). One of the two patients with severe sporadic cerebellar cortical atrophy, aged 29 yrs (patient 14), was still able to walk whereas the second patient (patient 15), aged 49 yrs, was wheelchair-bound.

Seven age-matched normal subjects served as controls. Mean age was 49 yrs (range 34 – 63 yrs; three females and four males). Informed consent was obtained from all patients and normal subjects.

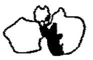
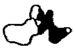
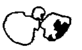
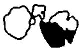
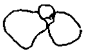
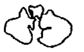
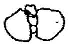
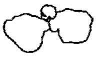
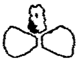
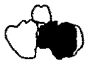
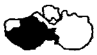
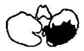
Experimental design

Saccadic and slow eye movements were first carefully tested using bedside methods. In addition, horizontal and vertical eye movements were recorded (Table 1) with d.c.-coupled, bitemporal electro-oculography (EOG) using skin electrodes. Signals were low-pass filtered (30 Hz cut-off frequency) and written out on a rectilinear 6-channel oscillograph for further analysis. Subjects did not wear their corrective glasses or contact lenses during testing. The fixation light was clearly visible to all subjects. Horizontal and vertical saccades were made spontaneously and on command in darkness and in response to step displacement (8 – 20 deg from the primary position) of a small target light (diameter 2.5 mm) at a distance of 90 cm in the primary position of the eyes.

Experimental protocol

The applied procedure (Fig. 1) was based on the experiments of Deubel *et al.* (1986) and Deubel (1989). The subject was asked to fixate continuously and to follow a small light spot (laser beam, diameter 2.5 mm) which was rear-projected onto a translucent tangent screen in an otherwise darkened room. There was no background structure. Viewing was binocular and head movements were restricted. The first task consisted of 50 – 100 trials of single target steps to each side to determine basic parameters of the saccadic reaction. The target light was stepped in rapid succession. The time (between 1 s and 4 s) and location (between 8 deg and 20 deg eccentrically to the primary position) of the target's steps were selected randomly. The actual experiment consisted of a sequence of 160 – 200 trials of double steps to each side. In these trials the primary saccade triggered an additional intra-saccadic target displacement which occurred in the direction opposite to the first step. The time interval between the first and second target step was therefore dependent on the latency of the primary saccade which ranged on average between 200 ms and 250 ms. An analogue electronic circuitry detected the primary saccade and triggered the signal to move the target light. The target light jumped to its second, final position well before the primary saccade was completed. The absolute amount of the intra-saccadic displacement (39% and 50% of the initial target step for normal subjects and 39% for patients) was variable and given by the amount of the first target step. The sequence of these double target steps trials formed the adaptation period (adaptation in Fig. 1). The intra-saccadic target

TABLE 1 SUMMARY OF DIAGNOSIS, LOCATION AND EXTENT OF LESIONS FOR PATIENTS 1–13

Case	*Age (yrs)*	*Sex*	*Diagnosis*	*MRI lesion level of most extensive pathology*	*Tested after (mths)*
1	43	M	W, C	r  l	15
2	50	M	W, C		50
3	53	M	W, C		21
4	68	M	W, C		60
5	64	M	W		5
6	61	M	W	CT	60
7	49	M	W		39
8	38	M	W		28
9	61	M	W		15
10	33	F	W		12
11	48	M	C		22
12	47	M	C		8
13	64	F	C		4

Magnetic resonance imaging of patients 2, 7, 11 are shown in Fig. 1A–I in Waespe and Wichmann, 1990. W = Wallenberg, C = cerebellar; r = right; l = left

step induced a consistent post-saccadic position error which had to be corrected. Initially, errors were large and corrected by correction saccades. However, as shown by Deubel *et al.* (1986) in normal subjects, only 150–200 trials are needed to adaptively change the gain of the primary saccade. This adjustment of saccadic amplitude is direction-specific (Miller *et al.*, 1981; Deubel *et al.*, 1986). After this sequence of double target steps, 30–50 control trials with single target steps were given again without intra-saccadic displacement of the target. Subjects were not able to recognize the experimental procedure (McLaughlin *et al.*, 1968; Miller *et al.*, 1981). Thus, it is very unlikely that the effects described below were obtained by volitional control (Hallett, 1978).

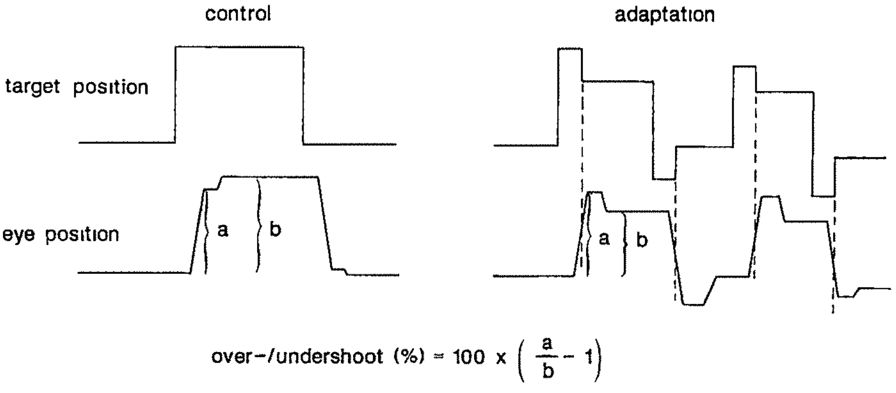


FIG 1 Sketch of the experimental sequence, before and after the adaptation paradigm single target steps (control) were given The adaptation paradigm consists of alternating double target steps The target was set back after the first step and this second step was triggered by the primary or main saccade made in response to the first target step One or several correction saccades were made for final fixation of the target (*see* Fig. 9). The final target position served as the starting position for the next trial into the opposite direction. Double target steps were delivered in the same session to both directions.

Pursuit eye movements during visual-vestibular interaction were also tested with sinusoidal stimulation (period 4 s, peak velocity 55 deg/s for smooth pursuit eye movements, and 63 deg/s for vestibular stimulation). We refer for details of these latter testings to our previous paper (Waespe and Wichmann, 1990).

Normal values

Normative values for the ability to change adaptively the amplitude of saccades as a function of trials using the 39% and 50% paradigm is shown in Fig. 7A for seven normal subjects and for five of these seven normal subjects, respectively. Values were averaged for blocks of 10 successive trials (Figs 7, 8, 10).

Data analysis

Saccade data such as velocity, amplitude, duration, latency and accuracy were extracted from the chart records by hand Velocity was measured by evaluating the hand-drawn slopes of the eye position trace and saccadic amplitude was measured as peak-to-peak change of eye position (Fig. 1). The inter-saccadic interval is defined as the time between the end of a saccade and the beginning of the following (corrective) saccade. To express the amplitude of saccades relative to the size of the target steps the equation in Fig. 1 was used. Overshooting of saccades is indicated by a positive sign; undershooting by a negative sign.

Magnetic resonance imaging studies

For details we refer to our previous report (Waespe and Wichmann, 1990). The location and extent of the ischaemic lesions in the patients are summarized in Table 1.

RESULTS

Single target step experiments

Normal subjects

Normal subjects regularly undershoot the target. Undershooting of primary saccades relative to the size of the target step is between −5% and −10% (Becker and Fuchs, 1969; Deubel *et al.*, 1986). In our seven control subjects undershooting was on average −6.7% (SD = 2.5%). Latency of the primary saccade to the step change of the target light was between 200 ms and 250 ms. In one representative subject this latency was on average 212.5 ms (SD = 22.5 ms). The duration of the interval between the end of the primary and the beginning of the secondary (first corrective) saccade was shorter. It was on average 162 ms (SD = 20 ms) when the primary saccades overshot the target (mean overshoot +17.5%) and 147 ms (SD = 22 ms) when the primary saccades undershot the target (mean undershoot −23%). As normal subjects very rarely have hypermetric or strongly hypometric saccades, measurements were taken from the initial trials during and after the adaptation paradigm when saccades were highly dysmetric. These values of the latency of correction saccades in or opposite the direction of the primary saccades correspond to those reported in the literature (Becker and Fuchs, 1969; Henson, 1978).

Patients

Figure 2 gives a typical example of the saccadic abnormalities found in five of our patients, all with Wallenberg's syndrome. Primary saccades made in response to an ipsilateral (lesion side) target step were often hypermetic whereas those made in response to a contralateral (normal side) target step were consistently hypometric (except in patient 10), forcing the patient to make multiple saccades for foveal fixation of the target.

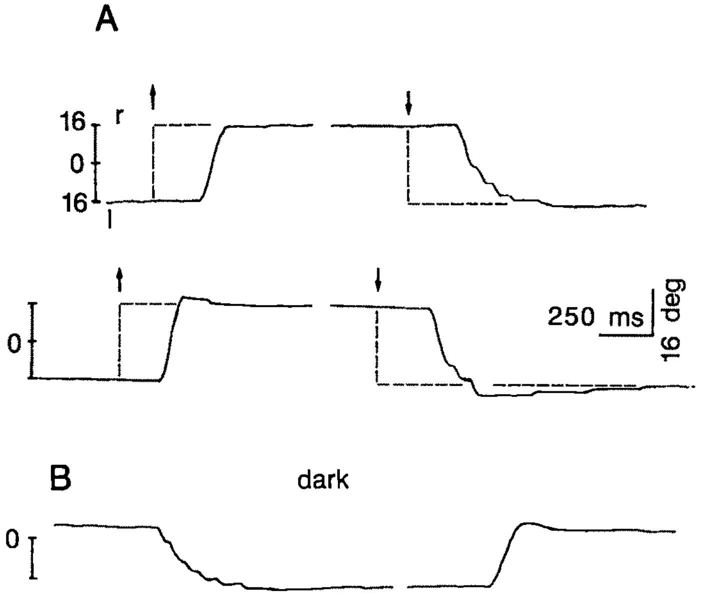


Fig 2. A, traced records of horizontal eye movements in patient 8 in response to ipsi- (right side, r) and contralateral (left side, l) single target steps. The position of the target is indicated by the broken line The target steps to the lesion side or normal side are marked by an upward or downward arrow, respectively. Ipsilateral primary saccades are slightly hypo- or hypermetric, contralateral primary saccades are hypometric, multiple corrective saccades in the direction of the primary saccade were made to refixate the target These corrective saccades could bring the eyes even into a position eccentric to the target, so that oppositely directed corrective saccades are made for refixation (second trace in A). B, in darkness, in response to remembered target steps of the same magnitude of ± 16 deg, a saccadic asymmetry was observed similar to that when the target was visible.

During the inter-saccadic intervals the eyes remained motionless or drifted slowly in the direction of the correction saccades (Fig. 2A). Corrective saccades could bring the eyes even to a position beyond the target. Thus, gaze could overshoot the target either with the primary saccade or with a cascade of correction saccades. If gaze overshot the target, backward directed corrective saccades were necessary for refixation. When patients were placed in total darkness and instructed to continue to refixate between the imagined locations of the previously visible target the asymmetry of saccades persisted (Fig. 2B).

Velocity and latency of primary saccades. In all patients the latency of primary saccades as well as mean peak velocities of saccades were within normal ranges (Becker and Fuchs, 1969; Baloh *et al.*, 1975). Figure 3 shows the velocity of primary and correction saccades as a function of amplitude in patient 8, this relationship being representative for all other patients. Average velocity of saccades of 30 deg amplitude was between 400 deg/s and 473 deg/s (average 435 deg/s, patients 6, 8–10, 12), and latency of the primary saccades ranged between 214 ms and 247 ms (average 225 ms, same patients).

Accuracy of target-directed primary saccades. Five patients (patients 2, 4, 6, 8, 10; Table 2) had asymmetric amplitudes of saccades: primary saccades in response to an ipsilateral target step were often hypermetric and larger than saccades made in response to a contralateral target step which regularly undershot the target. Mean over- and undershoot was +3.3% and −21.4%, respectively, for all five patients. Fifty-one percent of all primary saccades made by these five patients to the ipsilateral side, overshot the target by more than +2.5%. Patients 2, 4, 6, 8 made multiple corrective saccades in

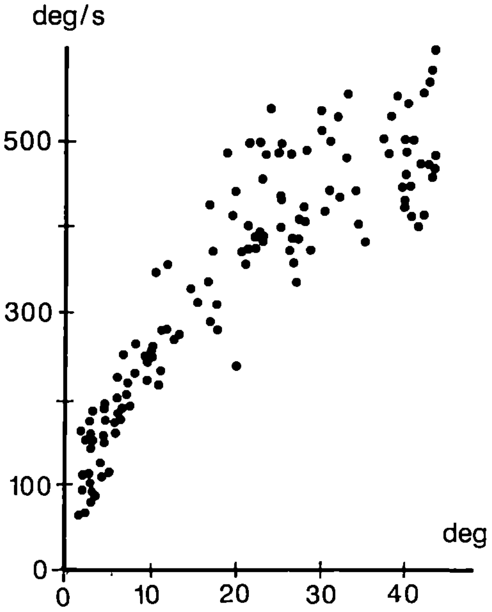


FIG. 3 Mean peak velocities (ordinate) of primary and corrective saccades as a function of amplitude (abscissa) in patient 8.

TABLE 2 ACCURACY OF PRIMARY SACCADES IN RELATION TO TARGET POSITION

	Ipsilateral			*Contralateral*		
		Overshoot >2.5%			*Overshoot >2.5%*	
Patient no	*Accuracy (%)*	*Primary saccade (%)*	*Multiple saccades (%)*	*Accuracy (%)*	*Primary saccade (%)*	*Multiple saccades (%)*
1	−16.5 (7.3)	–	19.0	−12.5 (7.0)	–	5.5
2	+2.0 (8.8)	43.5	–	−26.5 (13.5)	–	8.5
3	−16.0 (16.0)	8.5	15.0	−5.5 (6.5)	–	3.5
4	+5.0 (12.5)	57.0	9.0	−23.5 (17.0)	–	12.5
5	−6.0 (3.5)	<2.0		−9.5 (4.5)	–	–
6	+3.5 (11.0)	48.0	–	−27.0 (17.0)	–	–
7	−2.8 (5.8)	18.0	–	−3.5 (4.6)	6.5	–
8	+0.1 (12.0)	37.0	–	−23.0 (16.5)	6.5	36.5
9	−4.0 (5.8)	<2.0		−5.0 (4.3)	<2.0	
10	+6.0 (6.2)	61.5	–	−7.0 (5.3)	10.0	2.0
11	−7.5 (5.0)	<2.0		−5.5 (5.5)	<2.0	
12	−7.5 (3.0)	–	–	−9.5 (3.5)	–	–
13	−11.5 (7.8)	–	–	−10.5 (8.2)	–	–
Normal	−6.7 (2.5)	<2.0		−6.7 (2.5)	<2.0	

Mean of overshooting (+) or undershooting (−) with (1 SD). Percentage of target overshoot by more than +2.5% by the primary saccade or by multiple saccades.

response to contralateral target steps (Figs 2A, 5). In 15.2% of all trials with target steps to the contralateral side, either (seldom) the primary saccade or (most often) a cascade of corrective saccades brought the eyes lateral to the target, overshooting the target by more than +2.5%. In patients 1, 3, 7, although the target was undershot on average by the main saccades in both directions, in a considerable percentage of trials the target was overshot by the primary saccade or by a cascade of corrective saccades (Table 2). The distribution of the amplitudes of primary saccades relative to ipsilateral and contralateral target steps is shown for patients 8, 10 in Fig. 4. The relative accuracy of primary saccades and the percentage of target overshoot by more than 2.5% by single or multiple saccades is summarized for each patient in Table 2.

Duration of inter-saccadic intervals. Figure 5 shows the latency of correction saccades as a function of hypometria in patient 8. The latencies were increasingly larger when correction saccades brought the eyes near the target; the latency reached its largest value when the target position was overshot by the cascade of correction saccades and the following correction saccade was oppositely directed. Figure 6 summarizes for patients 8, 10 the latencies of the secondary (first corrective) saccade as a function of under- and overshooting of the primary saccade. As the primary saccades to the contralateral side rarely overshot the target for trials with single target steps, additional data for overshooting primary saccades were taken from the adaptation trials, i.e. from trials with double target steps. The latencies of the first corrective saccade were shorter for single target steps to which the patient habitually under- or overshot the target, than the corresponding latencies of corrective saccades which rarely occurred under normal conditions or which were induced during the adaptation trials. Note that latencies of correction saccades before (closed circles) and after (open squares) adaptation are comparable. Table 3 summarizes this finding for four patients. The average duration of the intervals between primary and secondary (first corrective) saccades was 214 ms

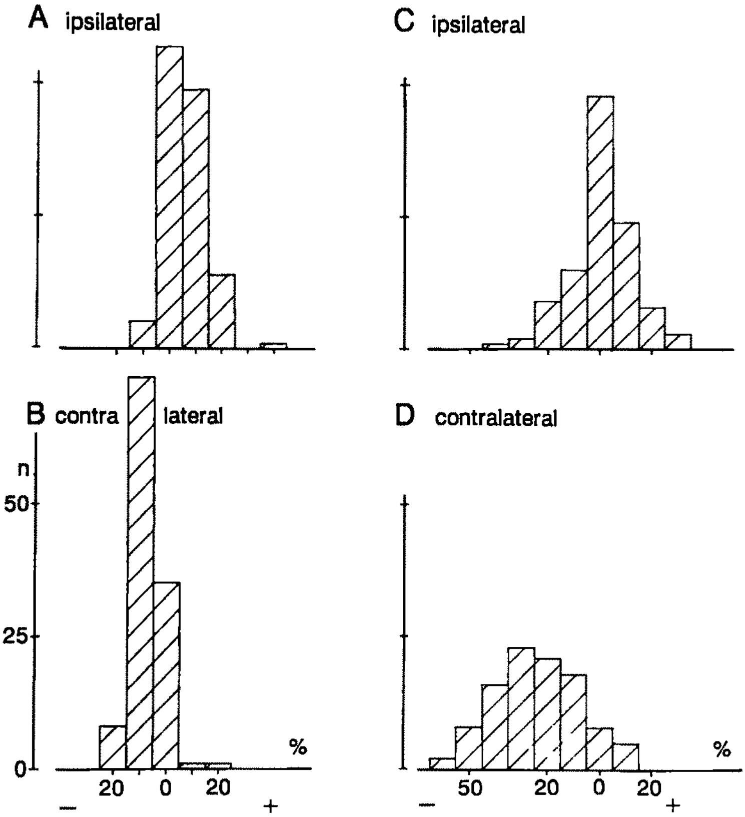


FIG 4. Distribution of the accuracy of primary saccades relative to the target step (abscissa) in patients 10 (A, B) and 8 (C, D). Bin width on the abscissa is ±5%. A, B mean overshoot for 126 ipsilateral primary saccades in A is +6% (SD = 6.2%), 61.5% of all primary saccades overshot the target by more than +2.5% and only 8% undershot the target by more than −2.5% (absolute value >2.5). Mean undershoot for 119 contralateral primary saccades in B is −7% (SD = 5.3%). Less than 1.5% of all saccades overshot the target by more than +2.5%, but 73.5% undershot the target by more than −2.5%. C, D, on average the 110 ipsilateral primary saccades in C were normometric (average 100.1%, SD = 12%), nevertheless 37% of all saccades overshot the target by more than +2.5% and 29.5% of all primary saccades undershot the target by more than −2.5%. In D the 101 contralateral saccades undershot the target on average by −23% (SD = 16.5%); 7.5% of all primary saccades overshot the target by more than +2.5%, and 90% undershot the target by more than −2.5%.

and 99.8 ms, respectively, when the primary saccades to the ipsi- or contralateral side were too short (undershooting between −2.5% and −30%). The difference of 114 ms in latencies may be explained by the fact that primary saccades regularly undershot the target for steps to the contralateral but not to the ipsilateral side. For target steps to the ipsilateral side undershooting rarely occurred under normal conditions. The intersaccadic interval was 135 ms and 228.5 ms, respectively, when the primary saccades to the ipsi- or contralateral side were too large (overshooting between +2.5% and +30%). Again, the difference of 93 ms in latencies is probably due to the fact that under normal conditions the primary saccades habitually overshot the target for steps to the ipsilateral but not to the contralateral side.

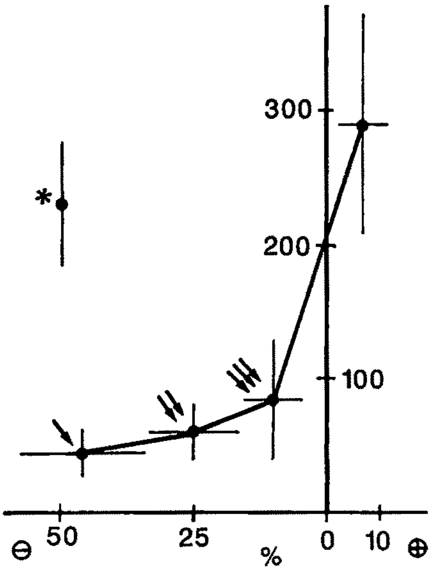


FIG. 5 Duration of inter-saccadic intervals (ordinate in milliseconds) between primary and first corrective saccades and between subsequent correction saccades in patient 8 as a function of under- and overshooting of saccades relative to the size of contralateral target steps. When the primary saccades undershot the target by −46% (SD = 11.5%) the mean duration between the primary and secondary saccade was 44 ms (SD = 18 ms) (marked by a single arrow). When the secondary (first corrective) saccade undershot the target by −24.9% (SD = 8.4%) the inter-saccadic interval betweeen the primary and secondary corrective saccade was 50.5 ms (SD = 21 ms) (double arrow). The secondary corrective saccade still undershot the target by −9.8% (SD = 5.6%), the latency between the secondary and tertiary corrective saccade was 84.5 ms (SD = 43 ms) (triple arrow). The tertiary corrective saccade overshot the target by +7% (SD = 4.3%), the average interval between the tertiary and quaternary corrective saccade, the latter directed opposite to the former, was 290 ms (SD = 82.5 ms). The latency of the primary saccades is 230 ms (SD = 46 ms) (marked with an asterisk).

Adjustment experiments

In these experiments the target was shifted intra-saccadically opposite to the initial first target step, resulting in a large overshoot of the primary saccade and thus in a target position error (*see* Patients and methods for details). This overshoot was corrected by one or several saccades in the opposite direction of the primary saccade.

Normal subjects

After 150−200 trials the amplitude of the primary saccades was adjusted relative to the final target position. This is shown in Fig. 7A (b, adaptation) as a decrease of the percentage overshoot relative to the final target position. Adjustment is complete for the paradigm with 39% target overshoot (filled circles in Fig. 7A). After the adaptation period control saccades made to single target steps undershot the target by −15% to −17% (c) as compared with control values of −6% to −7% before adaptation (a). The amount of adaptation in our normal subjects is quite similar to that found by Deubel *et al.* (1986). With 50% target overshoot (open circles) adaptation was not yet complete after 200 trials. Undershoot of control saccades after adaptation was between −20%

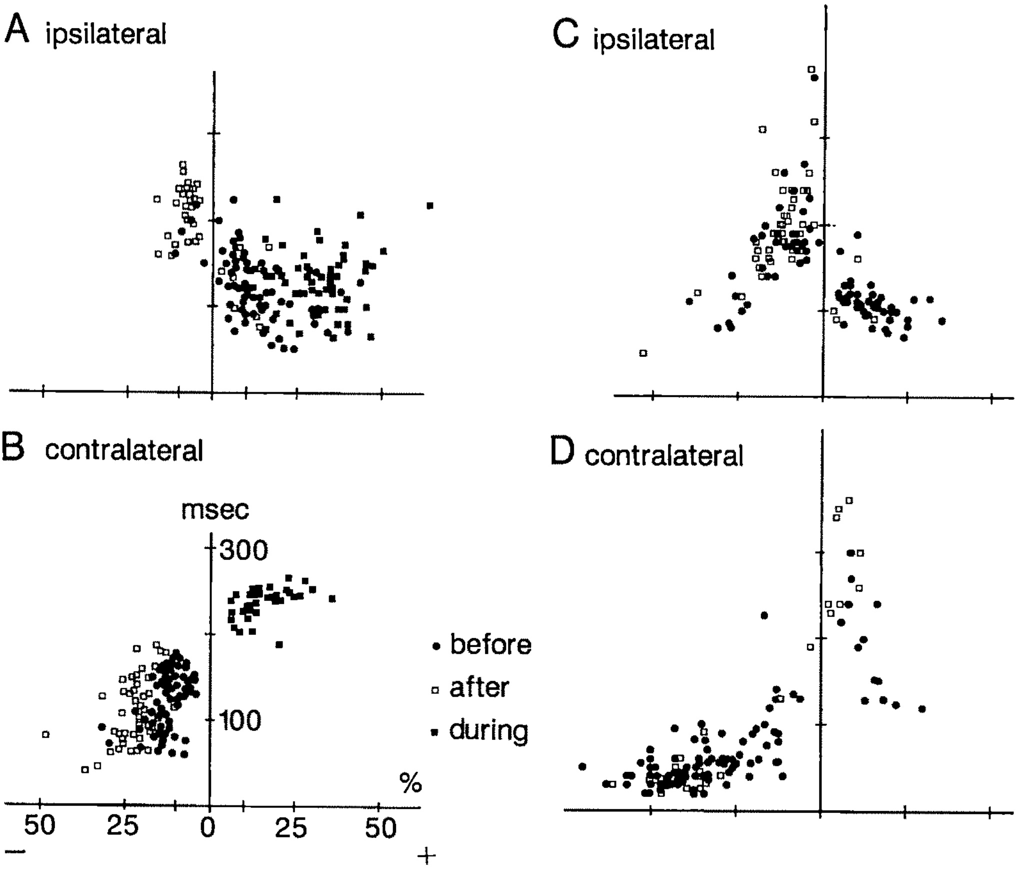


FIG. 6 Inter-saccadic interval (ordinate) between primary and secondary (primary correction) saccades as a function of over- and undershooting of the primary saccade (abscissa) in patients 10 (A, B) and 8 (C, D). Measurements were taken in trials before, during and after adaptation experiments. Note that when the primary saccades had the tendency to overshoot (A, C, for ipsilateral steps) or undershoot (B, D, for contralateral steps) the target under normal conditions (filled circles), the latency is shorter than in trials in which the primary saccades rarely or never over- or undershot the target (*see also* Table 3). Latency measurements in these instances were possible only in trials during (filled squares) and after (open squares) the adaptation paradigm.

and −22%. Adaptation also occurs during the control period in (c) with repetition of trials the amount of hypometria progressively decreases.

Patients

The adaptation paradigm of Deubel *et al.* had been applied so far to normal subjects only. To test for its sensitivity we applied it to two patients who suffered from sporadic cortical cerebellar atrophy. On the basis of experimental work cited in the Introduction no adjustment of saccadic eye movements was expected to occur.

Patients 14 and 15 with severe cerebellar cortical atrophy. Both patients had hypermetric saccades, a feature well known to occur in degenerative cerebellar disease. Postsaccadic drift was either not present or minimal. Mean saccadic overshoot was +5%

TABLE 3 DURATION OF INTERSACCADIC INTERVALS

		Undershooting −2.5% to −30%		*Overshooting +2.5% to +30%*	
Patient no		*Ipsilateral*	*Contralateral*	*Ipsilateral*	*Contralateral*
2	D	241.0 (36.5)	93.5 (61.5)	179.0 (71.5)	229.0 (26.5)
	Percentage	−11.0 (5.0)	−17.0 (7.0)	+14.0 (8.0)	+18.0 (5.5)
6	D	217.0 (37.0)	97.0 (30.0)	130.5 (20.0)	202.0 (44.8)
	Percentage	−7.8 (3.6)	−20.0 (6.2)	+8.8 (5.0)	+15.4 (6.3)
8	D	190.0 (50.0)	88.5 (46.5)	106.5 (27.0)	247.5 (76.5)
	Percentage	−12.5 (6.5)	−19.5 (7.7)	+13.0 (6.5)	+10.0 (7.1)
10	D	208.5 (30.2)	120.0 (35.0)	124.0 (36.5)	236.0 (17.9)
	Percentage	−8.0 (3.2)	−15.0 (6.1)	+14.7 (7.9)	+15.5 (6.3)
Mean	D	214.0 (21.0)	99.8 (13.9)	135.0 (31.0)	228.5 (19.3)
	Percentage	−9.8 (2.3)	−17.9 (2.3)	+10.1 (4.3)	+14.7 (3.4)

Mean duration of intervals between primary saccade and secondary (first correction) saccade, when the primary saccade is undershooting (between −2.5% and −30%) or overshooting (between +2.5% and +30%) the target for ipsi- and contralateral target steps Duration (D) in milliseconds (1 SD) and amount of under or overshooting in percentages (1 SD).

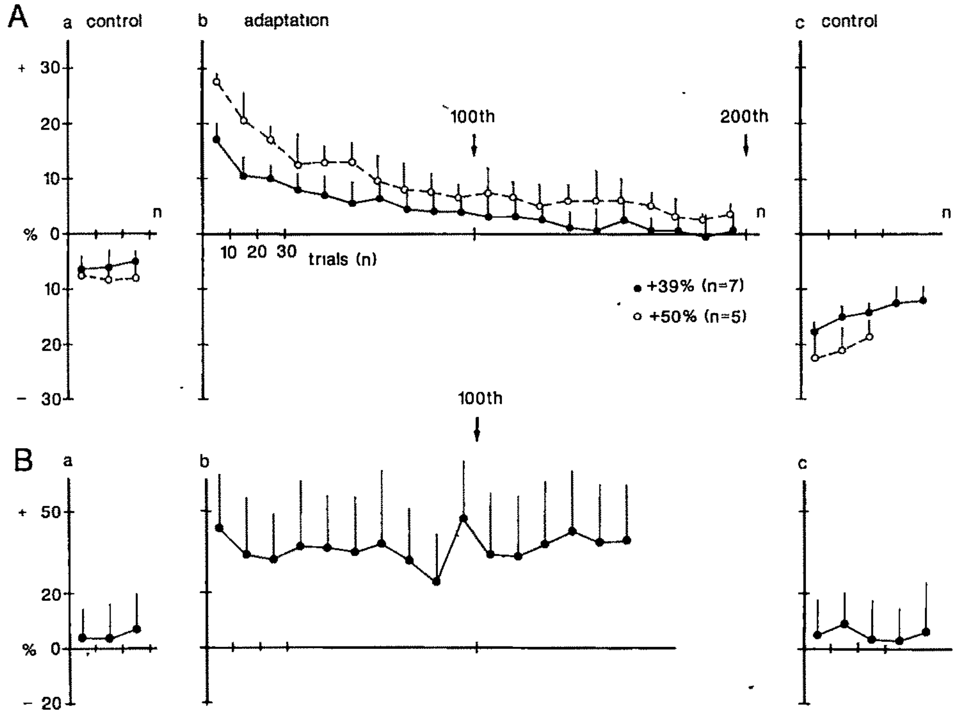


FIG. 7. A, relative over- and undershoot of primary saccades of normal subjects for the 39% target overshoot paradigm (filled circles; n = 7) and the 50% target overshoot paradigm (open circles; n = 5). Each data point represents the average (vertical bar is 1 SD) of 10 successive trials. In control trials before adaptation (a) the averaged undershoot is between −5% and −8%, after adaptation in (c) between −14% and −23%. B, saccadic adaptation in patient 14 with a sporadic cerebellar cortical atrophy. Average values with 1 SD are for trials into both directions Gain adaptivity of saccades is lost: there is no decrease in the amount of hypermetria during adaptation (b), and control values before (a) and after (c) the adaptation trials are similar.

(SD = 12%) in patient 14, and +16% (SD = 5.5%) in patient 15 in control trials. Both patients were unable to change the gain of their primary saccades during the adaptation paradigm. They made correction saccades in the direction of the second target step for refixation of the target during the whole period of adaptation. The latency between the primary, hypermetric saccade and the following correction saccade was 170 ms (SD = 20.5 ms, patient 14) and 165 ms (SD = 22.5 ms, patient 15). These values are in the range of those of normal subjects (162 ms, *see* section on normal subjects). As shown in Fig. 7B for patient 14, no decrease in the amount of hypermetria of primary saccades occurred during the adaptation trials (b). Loss of gain adaptivity was also demonstrated by unchanged amplitudes of saccades in control trials after the adaptation period. In patients 14, 15 the amount of overshoot after the adaptation trials (c) was +5.5% (SD = 13.5%) and +14% (SD = 5%), respectively, and thus identical to the overshoot in control trials before adaptation (a).

Patients with Wallenberg's syndrome. Patients 1 and 3 will not be discussed further. Their primary saccades to the ipsilateral side showed a large undershoot of more than −12%. The other eight patients were separated into two groups on criteria discussed below. Patients 5, 7, 9 constituted the first group and patients 2, 4, 6, 8, 10 the second group.

In Group 1 (patients 5, 7, 9), the patients had fairly symmetrical target-directed saccades (Table 2). In patient 7, 18% and 6.5% of all primary saccades overshot the target for ipsi- and contralateral steps, respectively. The curve in Fig. 8A suggests that these three patients had on average a decreased gain adaptivity for ipsilateral saccades. After 150−200 trials primary saccades still overshot the target by about +8% to +11%. Adaptivity for contralateral saccades was similar to that of normal subjects: after 150−200 trials of double target steps saccadic overshoot was minimal.

In Group 2 (patients 2, 4, 6, 8, 10) the saccades to the ipsilateral side overshot the target and saccades to the opposite side undershot the target in single target step trials (Table 2). The relative overshoot of ipsi- or contralateral saccades for the initial trials of the adaptation paradigm was greater or smaller than in control subjects (Fig. 8B). There was a tendency of the overshoot of ipsilateral primary saccades to decrease with increasing numbers of trials during the adaptation paradigm, but after 200 trials primary saccades still overshot the final target position on average by +20% to +25%. Control saccades to the ipsilateral side after the adaptation period slightly undershot the target, on average by −1% to −5%. Control saccades before the adaptation period overshot the target, on average by +1% to +2.5%.

In control trials the primary saccades undershot the target on average by about −16% to −20% (Fig. 8B) in the contralateral direction. The amplitude of primary saccades was further decreased by the application of double target steps: in control trials after the adaptation period the target was undershot on average by −26% to −29%. The gain of primary saccades to the contralateral side further decreased during the period of adaptation (b) despite the fact that primary saccades were on average hypometric in respect to the final target position (except for the initial 10 trials in Fig. 8B). However, during the whole period of adaptation the final target position was occasionally overshot by primary saccades and more often by cascades of correction saccades, similar to trials with single target steps. It is noteworthy that a further corrective saccade could occur in the direction of the first target step (and thus in the wrong direction) despite the fact

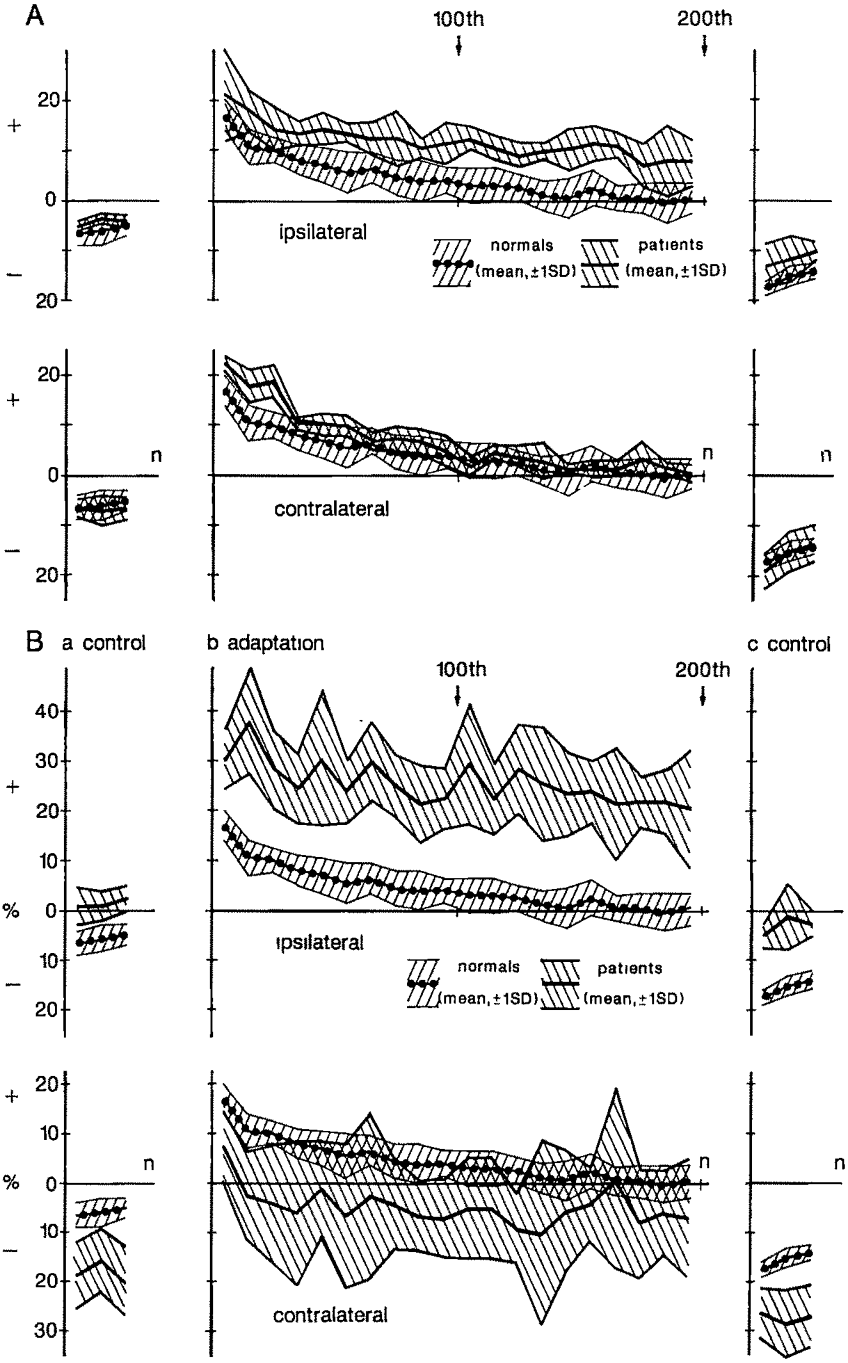


FIG. 8 Gain adaptivity of ipsilateral and contralateral saccades in patients 5, 7, 9 (A, heavy line), and in patients 2, 4, 6, 8, 10 (B, heavy line) for the 39% adaptation paradigm, compared with gain adaptivity of seven normal subjects (closed circles). Same display as in Fig. 7. Mean values with the 67% confidence level (dashed areas) are separated for ipsi- (first trace in A, B) and contralateral (second trace) saccades. For details *see* text.

that the target was overshot by the previous correction saccade (arrow in Fig. 9B, C). This overshoot transferred the target to the opposite side of the fovea. This kind of wrongly directed corrective saccades was, however, observed only during the initial trials of the adaptation paradigm.

Figure 10 shows gain adaptivity for patient 10. Between the first and second run of the adaptation paradigm there was a rest period of 5 min. A slight gain reduction for

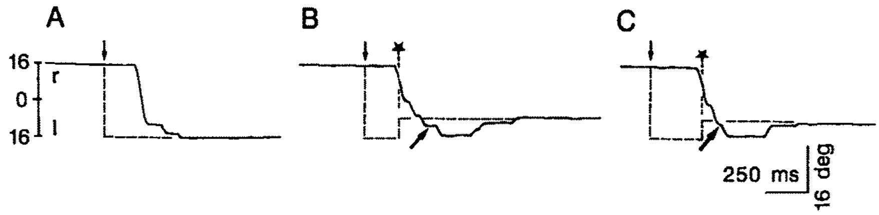


FIG 9. A, undershooting of the target in a single step trial in patient 8. B, C, double target step trials: a cascade of correction saccades overshot the final target position after the second target step. The position of the target is indicated by the dashed line, the first step is marked by a downward arrow, the second step, triggered by the primary, hypometric saccade, by an asterisk Note in B and C that, although the second (B) or first (C) corrective saccade has already overshot the final position of the target (arrow), a further corrective saccade is made in the direction of the first target step carrying the eyes even further away from the target.

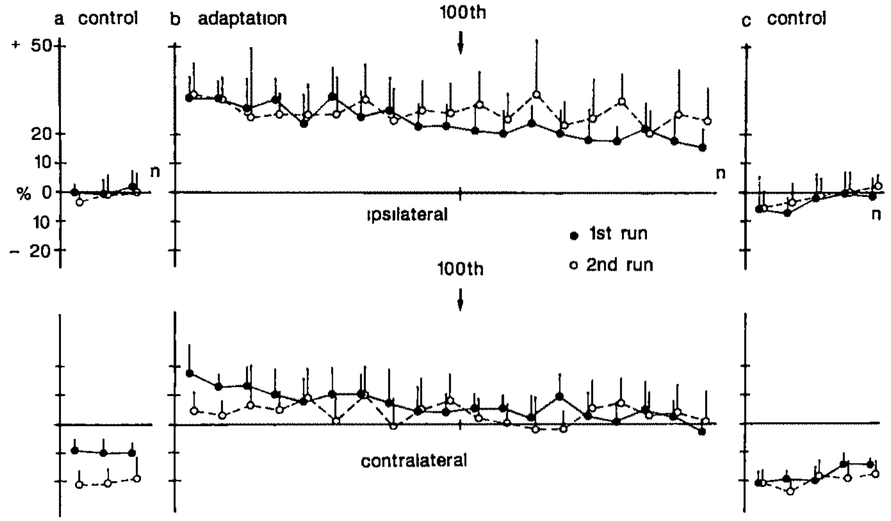


FIG 10. Effects of repeating the adaptation paradigm after a 5 min rest period in patient 10. Same display as in previous figures Note that there was a slight gain adaptivity of saccades to the ipsilateral side during the first run of the adaptation paradigm, but that a second run of 190 trials (open circles) did not further decrease saccadic overshoot This overshoot ranged for trials 160–190 between +16% and +22% in the first run, and +21% and +31% in the second run. Saccadic undershoot in control trials after adaptation (c) was 2–5% less than before adaptation (a) Saccadic adaptation of contralaterally directed saccades occurred but there was still a tendency of target overshoot during adaptation trials (b)

ipsilateral saccades occurred during the first run (filled circles) as can be seen from the control values after the adaptation period. The initial overshoot in control trials after a rest of 5 min during the second run (open circles) was similar to that of the first run (filled circles). During the second run of the adaptation paradigm, overshoot after 200 trials was still +25%. For contralateral directed saccades, gain adaptation was present and it was retained after a 5 min rest period. Control trials (open circles in a) before the second run of the adaptation paradigm were consistently less than the corresponding values before the first run (filled circles). Control values for single target steps in (c) after the first and second run of the paradigm were similar. Although a slight overshoot occurred during the second run of the adaptation paradigm, this overshoot was not sufficient to further decrease the amplitude of the first saccade. Similar results were obtained in patients 2, 7, 8.

Patients 11–13 with cerebellar ischaemia alone. Their capability for saccadic readjustment was normal. Adaptation occurred slightly faster than in control subjects: after 100 to 130 trials adaptation was already complete (not shown).

Additional observations

Saccadic lateropulsion. Gaze shifts in the vertical plane had a horizontal component of the trajectory in five patients (patients 2, 4, 6, 8, 10). With upwards directed saccades the trajectory deviated to the lesion side, and with downward directed saccades the trajectory deviated to the normal side. This kind of deviation was found in patients 2, 4, 6, 10. In patient 8 the trajectory of upwards saccades was deviated to the normal side and that of downward saccades to the lesion side. Deviation was dependent on the size of the vertical saccade, it ranged between 1 deg and 10 deg.

Amplitude of fast phases of vestibulo-ocular reflex (VOR). If goal-directed saccades to the ipsi- and contralateral direction were asymmetric, this asymmetry was also observed for fast phases during the VOR: fast phases directed to the ipsilateral side were larger than those to the contralateral side. As an example, in patients 2, 8, ipsilateral directed fast phases had an average amplitude of 16.6 deg (SD = 8.4 deg) and 7 deg (SD = 5.5 deg), respectively, contralateral directed fast phases had an amplitude of 5.2 deg (SD = 3 deg) and 1.6 (SD = 1.3 deg), respectively.

Gain of VOR, smooth pursuit (SP) and VOR suppression. Values were mostly abnormal in patients 2, 4, 6, 8, 10. Vestibulo-ocular reflex gain was between 1 and 1.43 (normal range 0.71–0.97); SP gain varied between 0.19 and 0.82 (normal range 0.84–0.97). Vestibular-ocular reflex suppression was abnormal in all patients with a low SP gain to stimulation into the same direction.

Time constant of eye position integrator. Patients 2, 4, 6, 8, 10 with overt saccadic dysmetria had the shortest time constant of the integrator in darkness, ranging between 2.3 s and 9.2 s (normal >15s).

Other oculomotor disturbances. Two patients (1, 6) had monocular torsional nystagmus of the ipsilateral eye in primary position or when looking to the lesion side. All other patients had no spontaneous nystagmus in the primary position. Three patients (4, 6, 8) had square wave jerks and three patients (2, 4, 6) had skew deviation with, in one patient (2), vertical double vision when looking towards the lesion side. In none of the patients did the eyes deviate tonically to one side in darkness.

DISCUSSION

Persistent saccadic dysmetria in Wallenberg's patients

Half of the patients with an ischaemic lesion in the lateral medulla oblongata, either with (patients 2, 4) or without (patients 6, 8, 10) involvement of the cerebellum, had overt saccadic dysmetria months or years after onset of their illness. All these patients also had horizontal deviation of the trajectory of vertical saccades, called saccadic lateropulsion (Kommerell and Hoyt, 1973). About 50% of the target-directed primary saccades to the lesion side were hypermetric; those to the normal side were, except in patient 10, mostly hypometric. The remaining patients also had signs of saccadic dysmetria. Although the gain of their primary saccades was on average within or below normal limits, in a considerable percentage of trials the target was overshot by a single (the primary) saccade or by multiple correction saccades. In normal subjects saccadic overshoot of a target is exceptional, at least for the range of target steps used in this study. In a cascade of correction saccades the inter-saccadic interval was dependent on the amount of undershooting of the initial saccade. Latencies could be as short as 50 ms, a value only rarely found in normal subjects under special conditions (Bahill *et al.*, 1975; Becker and Jürgens, 1979). Visually induced correction saccades in normal subjects have a latency above 110 ms (Becker, 1976; Henson, 1978; Becker and Jürgens, 1979). Thus, if the error of the primary, hypometric saccade is large, one or several corrective saccades occur with very short latency, too short to be induced by retinal error signals (Becker, 1976). Corrections of large position errors are therefore executed in the 'extraretinal mode' (Becker and Jürgens, 1979). By falling habitually much too short of the target with the main saccade, the direction and amplitude of the correction saccade would be known prior to onset. However, in our patients there seems to be uncertainty about the amplitude of the correction to be made because in many instances the cascade of correction saccades resulted in a target overshoot. An 'extraretinal' mode of programming short-latency correction saccades is also suggested by the observation that during the first trials of the adaptation paradigm a corrective saccade was often made in the wrong direction. This correction saccade carried the target even further away from the fovea to the other side of the retinal hemifield (Fig. 9). This retinal position error signal did not prevent the occurrence of a further corrective saccade in the wrong direction, probably because the visual error signal was first transmitted to the hemisphere contralateral to that which received the initial error signal elicited by the first target step. The necessary interhemispheric transfer probably prolonged the time of processing of the visual information by the saccadic system.

Cerebellar lesions and saccadic dysmetria

Saccadic dysmetria is a well-known feature in primates with cerebellar cortical or medial nuclei lesions (Ritchie, 1976; Selhorst *et al.*, 1976; Zee *et al.*, 1976; Optican and Robinson, 1980; Vilis and Hore, 1981). Saccadic reaction times and velocities are within normal limits as in patients with Wallenberg's syndrome. Most often, overshoot dysmetria occurs, but also a variety of patterns of dysmetria has been observed. In monkeys with bilateral vermal and paravermal lesions the pattern of dysmetria was eye position dependent. Saccades from an eccentric position towards the primary position were larger than those from the primary position towards eccentric positions (Ritchie, 1976). We did not test our patients for this kind of eye position-dependent dysmetria, saccades had to be made from the ipsi- into the contralateral hemifield and vice versa. Reversible unilateral lesions of medial cerebellar nuclei (nucleus fastigius and interpositus) induced horizontal deviation of vertical saccades to the lesion side (Vilis and Hore, 1981). Similar deviations of the trajectory of vertical saccades were observed in those patients with the lateral medullary syndrome who showed dysmetria of horizontal saccades. In the study of Vilis and Hore (1981), 75% of all ipsilateral goal-directed saccades were hypermetric whereas 40% of the contralateral saccades were equally hypo- or hypermetric. The suggestion was made that the cerebellum is involved in tuning some internal estimate of extraocular muscle strength (Vilis and Hore, 1981). Overestimation of muscle strength would result in hypometric saccades. Efference copy signals of saccadic performance would wrongly signal that the target is reached before the eyes actually get there (depending upon muscle strength). As a consequence the saccadic eye movements would be terminated too early. Hypermetric saccades occur with underestimation of muscle strength and saccades are terminated too late. It is premature to speculate on the mechanisms which underly enduring saccadic dysmetria in patients with Wallenberg's syndrome. The simplest model of the saccadic system assumes that the magnitude of a saccade is determined by a comparison between desired eye or target position and a predicted actual eye position based on efference copy (Robinson, 1981). This comparison results in an eye position error signal. If the eyes are driven by this signal until the error signal becomes zero the eyes would at this point stop moving. If the saccadic system computed the location of the target in relation to space, the initial eye position before a saccade would also be information essential to the functioning of the saccadic system. Dysmetria may result from misrepresentation of the error signal or of eye or target position. When saccadic dysmetria is permanently manifest, it could also mean that adaptive mechanisms can no longer compensate for it as dysmetria has become too large, or that the mechanisms themselves are disturbed. The adaptive process may be disrupted in any of its component parts, i.e. the 'error' inputs that signal the need for adaptation, the central networks that calculate the necessary readjustments or the outputs that mediate corrective command signals to the premotor structures. Cerebellar lesions, more specifically of the posterior vermis, could cause inappropriate gain adjustments of saccadic amplitudes resulting in dysmetria (Robinson, 1975; Zee *et al.*, 1976; Optican and Robinson, 1980). Even with practice, cerebellar patients never learned to correct their dysmetria (Zee *et al.*, 1976).

Rapid saccadic gain adaptivity

The two patients with cerebellar cortical atrophy not only had overshooting dysmetria, but their capability of gain adjustment of the hypermetria, if it was further increased artificially by using double target steps, was lost. This supports the idea that cerebellar cortical structures in humans are involved in gain adjustment of saccadic eye movements.

Although a direct comparison of the capability for saccadic gain adaptation in patients with Wallenberg's syndrome with that in normal subjects is difficult because the amplitudes of the control saccades are different in both groups, the results suggest impaired saccadic gain adaptivity in the patient group. The amplitude of ipsilaterally directed hypermetric saccades did decrease adaptively to some degree, but after delivery of 200 double target steps to each side, primary saccades were still hypermetric. Figure 7A suggests that the drive for saccadic readjustment is stronger with larger target position errors. Due to the already hypermetric saccades in single target step experiments, the target position error was initially larger in patients 2, 4, 6, 8, 10 than in normal subjects in the 39% double target paradigm (Fig. 8B) but comparable to the error in the 50% paradigm in normal subjects (about 30% overshoot for the initial 10 trials, Figs 7A, 8B). Yet, after 200 trials saccadic overshoot was still between 20% and 25% in patients as compared with 5% in normal subjects. The amplitude of contralaterally directed hypometric saccades is not increased adaptively as it would be expected from the experiments of Deubel *et al.* (1986) in normal subjects. These authors showed that the amplitude of undershooting primary saccades slowly increased adaptively if the second target step was *in* the direction of the first target step. The further decrease in amplitude of already hypometric primary saccades observed in our patients may be due to the occasional target overshoot either by the main saccade or by a cascade of correction saccades. Deubel *et al.* (1986) showed that the amplitude adaptively decreased even though only one-third of all primary saccades overshot the target in the double target-step paradigm. The occurrence of only a certain percentage of hypermetric saccades is sufficient to prevent hypermetria. As mentioned above, the persistent saccadic dysmetria in patients with Wallenberg's syndrome already suggested that gain adaptivity of saccadic eye movements may be impaired; the results of the examination of saccadic gain adaptivity itself are in agreement with that idea. This kind of adaptive control which is accomplished within minutes has been called *rapid* saccadic adaptation as opposed to *gradual* saccadic adaptation which takes hours or days to develop (Optican, 1985). By the former mechanism the saccadic system tries to maintain hypometria, and by the latter it compensates for post-saccadic ocular drift. A note of caution should be made. We recorded the movements of the two eyes as a single movement. Vilis *et al.* (1983) demonstrated that the degree of saccadic dysmetria induced by medial cerebellar nuclei lesions is different in the two eyes. Thus it is possible that the degree of saccadic dysmetria and of impaired gain adaptivity is different in the two eyes of our patients.

Suggested functional-anatomical correlations

Functional-structural correlations in the lateral medullary syndrome are hypothetical. In a previous paper we suggested that olivo-cerebellar pathways are likely to be interrupted (Hoyt and Frisén, 1975; Waespe and Wichmann, 1990). After having crossed in the brainstem, these fibres run in the inferior peduncle to the cerebellum and are most probably damaged in the lateral aspect of the medulla. It is important to note that the lesion in Wallenberg's syndrome is unilateral, interrupting olivo-cerebellar fibres to the ipsilateral cerebellum only. Saccadic dysmetria has not yet been observed in animals after destruction of the inferior olivary nucleus or inactivation of its fibres. Animals, however, were not trained to fixate between different targets. Patients with inferior olivary nucleus degeneration were not reported to have saccadic dysmetria (Ridley *et al.*, 1987). None of our patients had opsoclonus or palatal myoclonus which may be observed with inferior olivary nucleus degeneration. How disruption of olivo-cerebellar fibres interferes with the mechanism of saccadic gain adaptivity may only be speculated upon. Disrupted olivo-cerebellar fibres may transmit retinal error signals and/or signals representing eye position or muscle strength to cerebellar Purkinje cells. These signals which provide information about the actual or predicted performance of the saccadic system would then no longer reach cerebellar cortical circuits. Alternatively, chronic inferior olivary nucleus deafferentation results in a reduced efficacy of the inhibitory action of Purkinje cells, decoupling them functionally from their target cells in the cerebellar nuclei (Karachot *et al.*, 1987). Thus, even if cerebellar cortical circuits received normal mossy fibre input signals, inhibitory Purkinje cell action on their target cells which are involved in the mediation of amplitude command signals of saccades would be less effective due to the mere disruption of climbing fibre input activity, irrespective of the specific information carried by the damaged climbing fibres. Animal experiments are generally in support of a crucial role for the inferior olivary nucleus and its fibres in the acquisition and retention of adaptive effects on motor responses such as the vestibulo-ocular reflex (Ito and Miyashita, 1975; Robinson, 1976; Haddad *et al.*, 1980; Demer and Robinson, 1982; Ito, 1982) or the conditioned eyelid response (McCormick *et al.*, 1985; Mauk *et al.*, 1986).

Conclusion

Clinical and experimental evidence support the notion that cerebellar processes, either intrinsic to the cerebellar cortex or involving its input and output pathways, are involved in adaptive control of motor responses. The results of the present study suggest that in Wallenberg's syndrome, structures or pathways in the lateral medulla which are necessary to continuously modulate saccadic gain, whether by processing or transferring visual or eye position signals or efference copy signals, are lesioned. The observation that saccadic gain adaptivity induced by retinal position error signals is impaired, suggests that the disrupted structures or pathways process or transmit visual error signals which are necessary for adaptive gain control of saccades by the cerebellum. Although precise structural-functional correlations are not possible in Wallenberg's syndrome, we suggest that the persistent saccadic eye-movement abnormalities are most likely the effect of disrupted olivo-cerebellar fibres which run in the posterior peduncle to the cerebellum.

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ABNORMALITIES OF PREDICTIVE SACCADES IN HEMI-PARKINSON'S DISEASE

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SUMMARY

We studied reflexive and predictive saccades by direct current electro-oculography in nine patients with mild hemi-Parkinson's disease (hemi-PD) and in 16 age-matched controls. In five patients, the neurological syndrome was predominant on the right side of the body (RPD) and in four patients, on the left side (LPD). Reflexive saccades were elicited in response to the random appearance (timing and location) of a light-emitting diode (LED). Predictive saccades were elicited by alternatively illuminating LEDs at 10 degrees right and left, at various fixed frequencies (0.25–1 Hz).

In the reflexive task, latency and amplitude of the saccades were normal in both PD groups In the predictive task, mean saccade latency was not significantly different between patients and normals but there were two significant abnormalities in timing: first, but only in LPD, a directional asymmetry in latency (left greater than right, e.g. at 0.25 Hz, mean difference of 90 ms); secondly, especially in RPD, an abnormal tracking pattern, reflected by more variability of the mean value (for each group of patients) of saccade latency at each point in time, throughout a period of tracking at a given frequency. Predictive saccades were also strongly hypometric in both PD groups but especially in LPD (e.g. for rightwards saccades: controls = 19 degrees, SD = 1.6; LPD = 14 degrees, SD = 2.7; RPD = 15.7 degrees, SD = 2.3). These defects in saccadic timing and amplitude during predictive tracking were most salient at low frequencies. While these defects were largely bilateral, our findings suggest slightly different contributions of the right and left cerebral hemispheres to the spatial and timing components, respectively, that comprise optimal predictive saccadic behaviour.

INTRODUCTION

Parkinson's disease (PD) is a degenerative disorder characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC). In addition to a somatomotor syndrome consisting of tremor, rigidity and bradykinesia, there are also abnormalities of eye movements (Kennard and Lueck, 1989; Leigh and Zee, 1991). In patients with mild PD, the saccades are delayed and hypometric in tasks that primarily reflect internally generated volitional motor programs, such as the response to targets moving in a predictable fashion (Bronstein and Kennard, 1985; Nomura *et al.*, 1986; Crawford *et al.*, 1989*b*; Kennard and Lueck, 1989; Lueck *et al.*, 1990). Saccades are less impaired in tasks that test reflexive types of behaviour such as externally triggered responses to novel visual stimuli.

Recent electrophysiological studies in the basal ganglia and in the frontal lobes of monkeys have linked both of these structures to the generation of more volitional saccades, in the context of learned, remembered or anticipated behaviour (Hikosaka and Wurtz, 1983*a,b,c,d*; Bruce and Goldberg, 1985; Deng *et al.*, 1986; Joseph and Barone, 1987; Goldberg and Segraves, 1989; Hikosaka and Wurtz, 1989; Hikosaka *et al.*, 1989*a,b,c*).

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Clinical studies of patients with PD and of patients with Huntington's disease (HD) also suggest that the frontal lobes and basal ganglia participate in the control of voluntary saccades (Bronstein and Kennard, 1985; Lasker *et al.*, 1987, 1988; Crawford *et al.*, 1989*a,b*; Lueck *et al.*, 1990; Tian *et al.*, 1991).

In the present study, we recorded saccadic eye movements in patients with mild hemi-Parkinson's disease (hemi-PD), using a predictive tracking paradigm to learn (i) if there are directional asymmetries in predictive behaviour and (ii) if the right and left cerebral hemispheres make different contributions to the spatial and the temporal aspects of predictive tracking. Our results confirm a deficit in predictive saccadic tracking in hemi-PD, especially at low frequencies. Surprisingly, the ocular motor deficits were largely bilateral in spite of the striking somato-motor asymmetry. Nevertheless, there was some asymmetry in the ocular motor abnormalities suggesting that there may be a difference between the contributions of the left and right cerebral hemispheres to both the temporal (timing) and the spatial (location) components necessary for generating optimal anticipatory responses during a predictive tracking task.

METHODS

Subjects

Nine right-handed patients with hemi-PD (four females, five males; mean age 59 yrs, range 51–69 yrs) and 16 right-handed, age-matched normals (11 females, five males; mean age 54 yrs, range 29–70 yrs) were investigated (Table 1). The patients were all followed in the movement disorder clinic and the age-matched normals were a mixture of spouses of patients and hospital employees and their relatives. All the patients were clinically examined by a neurologist (S.R.) and the PD disability was scored using the New York University rating scale with a maximal score of 52 (Lieberman, 1974). A score of 0 to 4 is assigned to the following abnormalities: tremor, rigidity, bradykinesia, gait and postural stability. Bradykinesia was evaluated using timed finger and foot tapping. All subjects gave informal consent to participate in this study. The patients were divided into two groups according to the affected side of their neurological syndrome: five patients had a right hemi-PD (RPD) and four patients a left hemi-PD (LPD).

TABLE 1. CHARACTERISTICS OF PATIENTS

	PD patients	*Sex*	*Age (yrs)*	*Illness duration (mths)*	*New York University disability (max. 52)*	*Tremor Max./side = 8*		*Rigidity Max /side = 8*		*Bradykinesia*	*Anti-PD medication*
						R	*L*	*R*	*L*		
RPD	M A R	F	66	18	9	6	0	2	0	BL	
	L U.T.	F	55	119	5	2	0	2	0	UL*	Sinemet
	P.J.C.	M	51	40	13	3	0	4	2	UL**	Artane
	B.A.K.	F	56	56	4	0	0	3	0	UL**	Sinemet, Eldepryl
	R.K.	F	52	32	10	0	0	4	2	UL	
LPD	A.D.L	M	69	76	15	0	5	2	4	UL*	Sinement, Artane
	L.H.	M	65	71	10	0	3	1	3	UL**	Sinemet, Eldepryl, Amantadine
	Q L D.	M	63	47	8	0	3	1	3	BL	Sinemet
	S.E.V.	M	57	9	7	0	3	1	2	UL**	Sinemet

Tremor was evaluated at the hand and rigidity at the elbow. Bradykinesia was scored on the basis of the timed-finger and foot-tapping tasks. * = mild; ** = severe asymmetry; UL = unilateral; BL = bilateral.

Apparatus and eye-movement recordings

The subjects were seated in a dark room in front of an arc (radius 123 cm) supporting light-emitting diodes (LED). The target lights subtended 0.1 degree of visual angle and were located at 10, 20 and 30 degrees right and left from the centre (0 degree). Head movements were restricted by a chin rest.

Horizontal saccades were recorded from the right eye by direct-current electro-oculography. Eye blinks were monitored by vertical eye movements recordings. Electro-oculography signals were amplified and filtered with a low-pass analogue filter (40 Hz). The filtered analogue signals were digitized at a sampling rate of 100 Hz by computer and saved for off-line analysis.

Procedure and paradigms

Both non-predictive and predictive saccades were tested.

Non-predictive paradigm (non-predictive stimulus). Each trial began with fixation of the centre LED. A peripheral LED was illuminated and simultaneously the centre LED was switched off and a non-localized beep, 100 ms in duration, sounded. The peripheral LED was presented at a random time (1400–2400 ms), direction (right or left) and amplitude (10, 20 or 30 degrees). Subjects were told to move their eyes towards the peripheral light as quickly and accurately as possible.

Predictive paradigm (predictive stimulus). Targets alternated between two fixed locations (10 degrees right and left) at various fixed frequencies in the following sequence: 0.25, 0.5, 1, 0.33 and 0.75 Hz. For each target frequency, 25 cycles of responses were collected. Subjects were told to move their eyes in time with the movement of the light.

Data analysis

An interactive computer program was used for saccade analysis; the accuracy of saccade identification was verified by visual inspection of each trial on a video monitor. For the non-predictive paradigm, saccades were calibrated independently for each eccentricity of targets. For the predictive paradigm, separate calibrations were obtained for each frequency of target motion. This calibration procedure minimized inaccuracy due to any fluctuations in the amplitude of the corneo-retinal potential.

For the non-predictive paradigm, the latency and the amplitude of the initial saccade in response to the target stimulus were computed.

For the predictive paradigm, saccades initiated within 100 ms of, or prior to, the target jump were classified as anticipatory. A negative latency indicated that the saccade was initiated before the target jumped. At each frequency of target motion, responses were divided into two groups: the anticipatory and the non-anticipatory saccades. The percentage of anticipatory saccades was calculated. For all of the predictive saccades (anticipatory and non-anticipatory saccades) and for each of the subgroup of non-anticipatory and anticipatory saccades, the analysis of latency and amplitude was performed independently for the rightwards and the leftwards responses.

A normal range for each parameter was established from the mean ±2 SD of the control group. Data were analysed using ANOVA, the Student's *t* test and the Mann-Whitney test, performed by the STATGRAPHICS software package. For ANOVA, within-group factors were the subject, the direction of the target and the frequency of the target motion. The dependent variables were the saccade latency and the saccade amplitude for the non-predictive and predictive paradigms, and also the percentage of anticipatory saccades for the predictive paradigm. A 95% confidence interval was used to establish statistical significance.

RESULTS

Non-predictive paradigm

The latency of visually triggered saccades was slightly but not significantly ($P > 0.05$, *t* test) increased in both directions for both hemi-PD groups (Table 2). The amplitude for 20 degree saccades was normal ($P > 0.05$, *t* test) for each hemi-PD group (Table 2) though one RPD patient (R.K.) showed an abnormal amplitude (11.1 ±3.2 degrees) for leftward saccades (control group: mean = 18.7 degrees; 2 SD = ±2.8).

TABLE 2 NON-PREDICTIVE PARADIGM LATENCY AND AMPLITUDE FOR RIGHTWARD AND LEFTWARD SACCADES IN CONTROL, RPD AND LPD GROUPS

			Control ($n = 16$)	*RPD* ($n = 5$)	*LPD* ($n = 4$)
Latency	Right	Mean	259.0	281.0	297.0
		SD	37.0	44.0	50.0
	Left	Mean	263.0	288.0	284.0
		SD	49.0	38.0	41.0
Amplitude	Right	Mean	18.2	17.9	17.0
		SD	1.3	0.7	1.3
	Left	Mean	18.7	17.1	17.7
		SD	1.4	3.4	1.3

SD = standard deviation; eccentricity of target = 20 degrees.

Predictive paradigm

Saccade latency

Although mean latency tended to be higher in patients versus controls (Fig. 1). there was no significant difference between the groups [group effects, $F(2,228) = 2.7$, $P = 0.065$, ANOVA].

Frequency effect. Latency depended on frequency in both patient groups and in the control group [frequency effect: $F(4,228) = 13$, $P < 0.0001$]. Mean latency was higher at low and high frequencies (0.25, 0.33, 1 Hz). This effect was greater in patients than in controls [control group: $F(4,139) = 11$, RPD group: $F(4,16) = 26$, LPD group: $F(4,12) = 39$] (Fig. 1).

Direction effect. The latency of saccades was consistently asymmetrical (left greater than right) only in LPD patients [direction effect in RPD: $F(1,16) = 2.2$, $P = 0.16$ and in LPD: $F(1,12) = 10.6$, $P = 0.0068$]. In the LPD group, the difference in latency between right and left saccades was greater at low frequencies and this effect was marked in two subjects (A.D.L. and S.E.V) [frequency/direction interactions: $F(4,12) = 4.5$, $P = 0.019$, and subject/direction interactions: $F(3,12) = 9.9$, $P = 0.0014$]. These findings are represented in Fig. 2.

Pattern of tracking. In the control subjects, the degree of anticipation depended upon the frequency of target motion; the lowest latencies, corresponding to the best anticipation, were at the intermediate stimulus frequencies (0.5, 0.75 Hz).

The pattern of predictive tracking in controls and in patients is illustrated for leftwards saccades in Fig. 3. We plotted the mean latencies, for each group, at each point in time, during the entire period of tracking at a given frequency. For instance, at 0.5 Hz in controls, mean saccade latency decreased in the first few trials and then became less variable and plateaued in the last 10 trials. However, at lower or higher frequencies, the values of saccade latencies showed more fluctuation.

Although the mean latency was not significantly different between the control group and the patient groups (based on the ANOVA analysis), the pattern of prediction was different (Fig. 3). The pattern in patients was more variable, especially at the lower and higher target frequencies.

To quantify these abnormalities, the last 20 mean values of the latencies throughout the period of tracking were analysed using the Mann-Whitney test. For leftward tracking

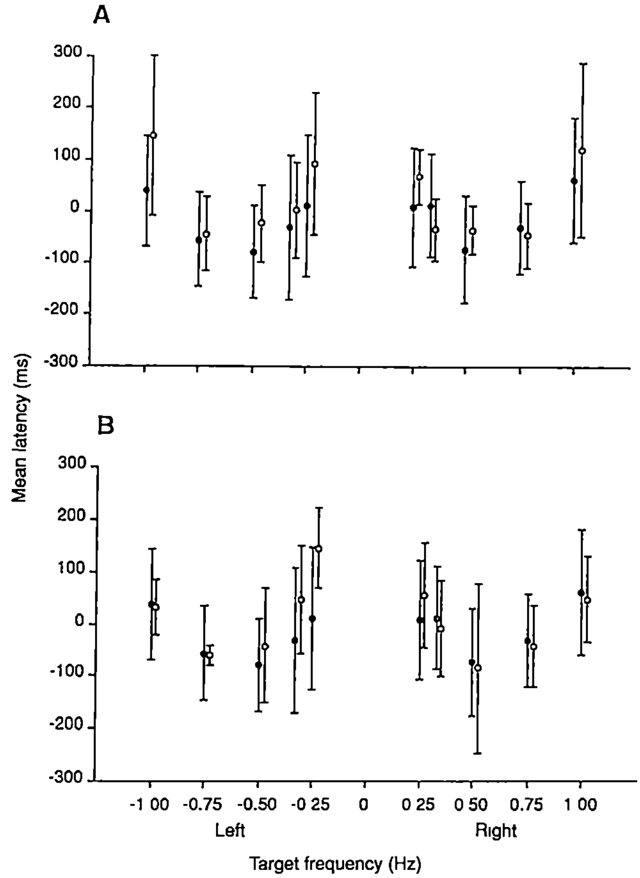

FIG. 1 Rightward and leftward saccade latencies during predictive tracking as a function of the target frequency. A, comparison between control (●) and RPD (O) group. B, comparison between control (●) and LPD (O) group

(*see* Fig. 3), there were significant differences compared with normal for both patient groups at low frequencies (0.50, 0.33, 0.25 Hz) and for RPD patients also at 1 Hz. For rightward tracking, there were significant differences ($P < 0.05$) only in RPD patients and only at some frequencies (0.25, 0.5, 1 Hz).

To summarize, the mean latency was normal in both patient groups. In the LPD group only, a directional asymmetry in latency was found between rightward and leftward saccades (left greater than right). Furthermore, especially in RPD, the pattern of tracking was abnormal as reflected in more variability of the mean values of saccadic latency over time, at a given frequency. These defects in latency were usually more pronounced at low frequencies.

Saccade amplitude

Patients showed a considerable decrease in saccade amplitude compared with normals [group effect: $F(2,228) = 42, P < 0.0001$, ANOVA]. The decrease in mean amplitude was greater in LPD than in RPD patients [$F(1,70) = 5$, $P = 0.026$] (Fig. 4).

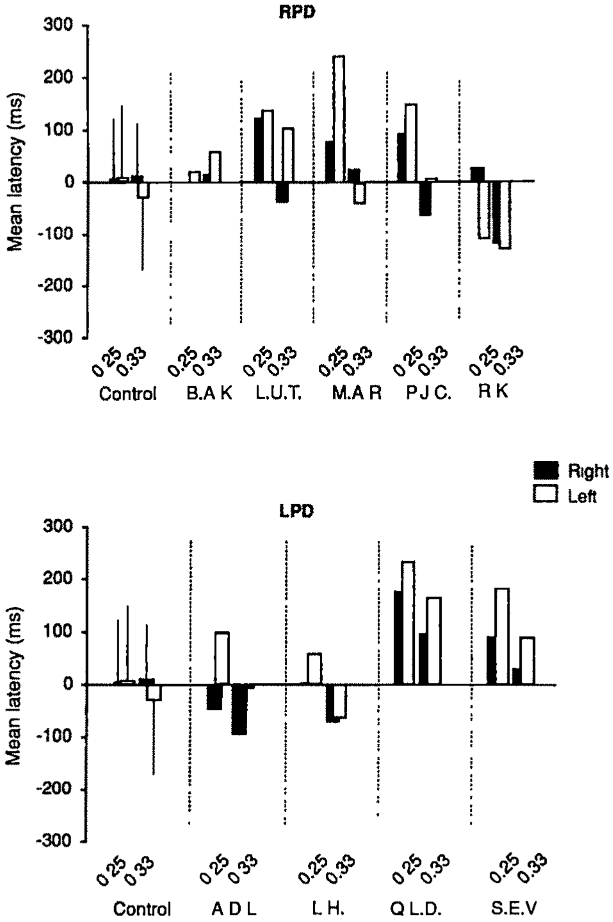

FIG. 2. Saccade latency during predictive tracking at the target frequencies of 0.25 Hz and 0.33 Hz The mean values are represented for the control group (with standard deviation) and for each individual of the patient group Right, rightward saccades, left, leftward saccades.

Frequency effect. The decrease in amplitude depended on frequency [frequency effect: F(4,228) = 11, $P < 0.0001$, ANOVA]: the lower the target frequency, the lower the saccade amplitude (Fig. 4).

Direction effect. The mean amplitude depended on saccade direction: the mean amplitude was slightly higher for rightward saccades in the control group and more so for leftward saccades in both hemi-PD groups. The effect of saccade direction on the accuracy within each patient group was analysed.

In RPD, all patients except one (R.K.) showed a significant difference in amplitude between right and left saccades (left greater than right); this effect was independent of the stimulus frequency (Fig. 5) [main effect of the direction factor F(1,16) = 17.7, $P = 0.0007$; subject/direction interactions F(4,16) = 19, $P < 0.0001$; frequency/direction interactions: F(4,16) = 1, $P = 0.38$].

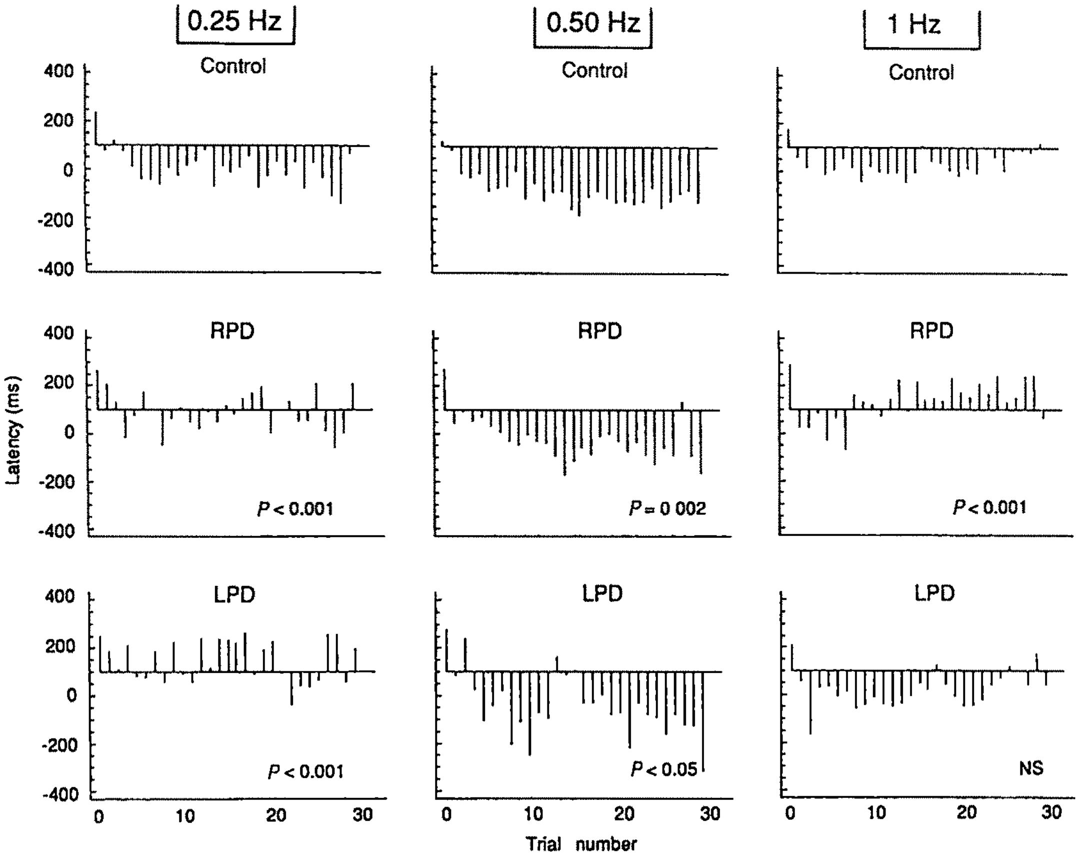

FIG. 3. Pattern for leftward saccades during predictive tracking at different target frequencies (0.25, 0.50, 1 Hz), for control, RPD and LPD group. Each vertical bar represents the saccade latency averaged at each trial for all the subjects in each group. Significant abnormalities ($P < 0.05$, Mann-Whitney test) in the variability of the tracking pattern are noticeable between the control and the patient groups. NS = no significance.

In LPD, all patients except one (S.E.V.) showed a difference in amplitude between right and left saccades (left greater than right); this effect was greater at low frequencies (Fig. 5) [main effect F(1,12) = 13, $P = 0.0035$; subject/direction interactions: F(3,12) = 8, $P = 0.003$; frequency/direction interactions: F(4,12) = 5.6, $P = 0.0087$]. To summarize: the mean amplitude for saccades in both directions was decreased in both groups of hemi-PD patients. This amplitude defect was more pronounced in LPD group and for the saccades directed to the right.

Amplitude variability. Saccade amplitude was highly variable over the 25 cycles of tracking, especially in the LPD patients. This variability was quantified by calculating the standard deviation of the mean value of amplitude for each subject over the last 20 trials, at each frequency (Fig. 6). Then, a mean value of the standard deviations was calculated for all subjects in each group. We found a significant increase ($P < 0.05$, student's t test) in the mean value of the standard deviations in both patient groups, mainly at low frequencies (Fig. 6).

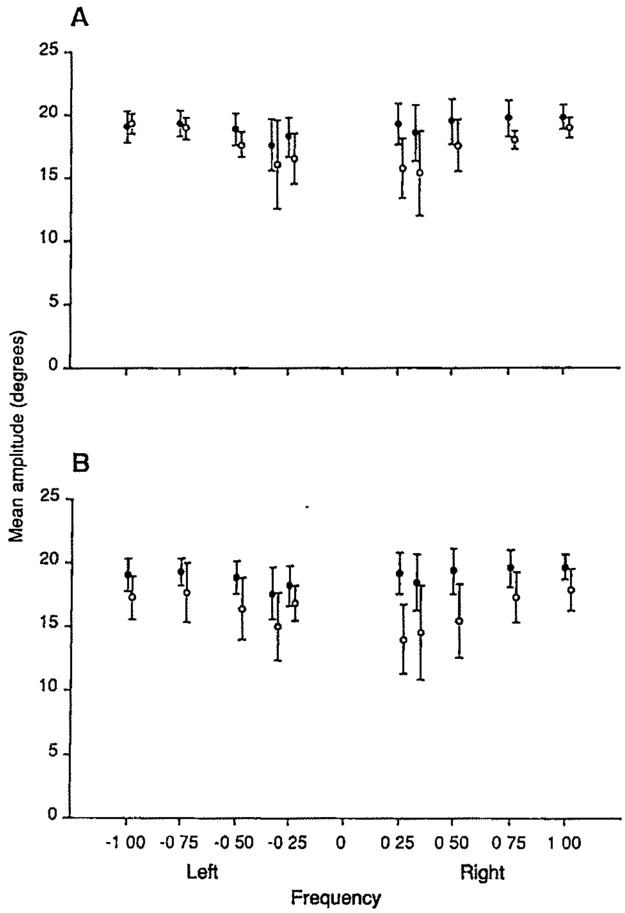

FIG. 4. Saccade amplitude during predictive tracking as a function of the target frequency A, comparison between control (●) and RPD (○) group. B, comparison between control (●) and LPD (○) group.

Anticipatory and non-anticipatory saccades. We further analysed saccades during predictive tracking by separating responses into anticipatory and non-anticipatory. An anticipatory saccade was defined as a saccade initiated with a latency before or within, 100 ms of the target jump. The percentage of anticipatory saccades in the patient groups was slightly but not significantly decreased with respect to the normal group [F(2,228) = 2.8, $P = 0.064$, ANOVA]. The percentage of anticipatory saccades depended on stimulus frequency in both patient and control groups [F(4,228) = 24, $P < 0.0001$]. As the frequency increased, the percentage increased, only to fall again when the frequency reached 1 Hz (Fig. 7).

There was, however, a highly significant abnormality in the amplitude of anticipatory saccades in both PD groups [group effect: F(2,225) = 67, $P < 0.0001$]; the amplitude deficit depended on frequency [frequency effect: F(4,225) = 21, $P < 0.0001$] and was more prominent in the LPD group [F(1,70) = 4.5, $P = 0.04$] (Fig. 8). The decrease in the amplitude of anticipatory saccade was independent of the response direction

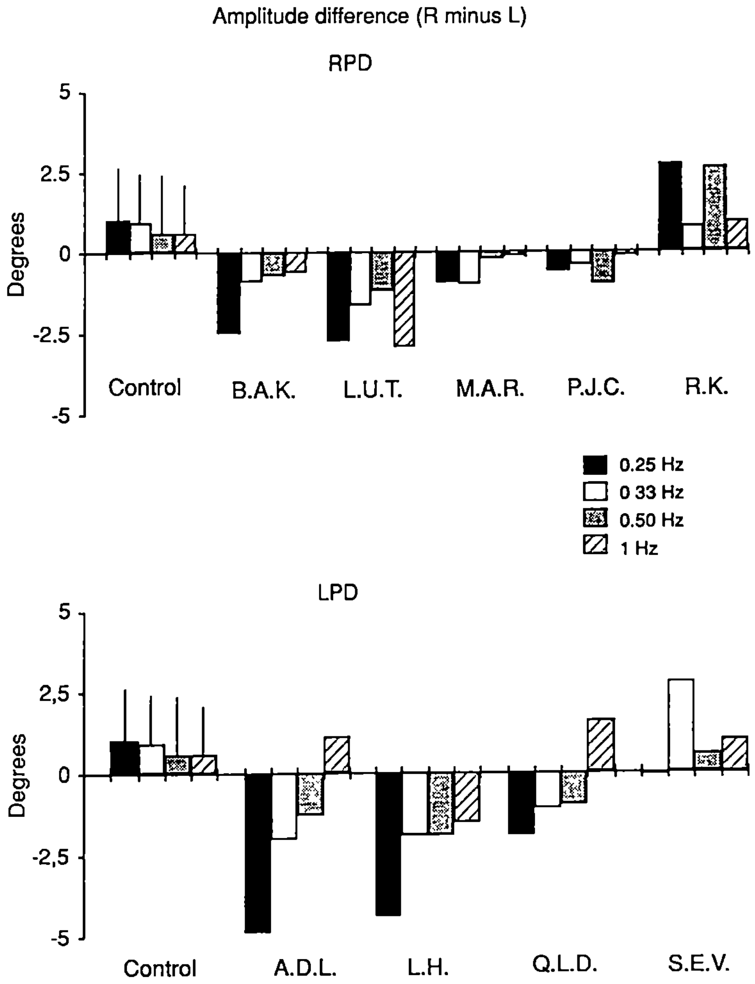

FIG. 5 Difference of amplitude between right and left saccades during predictive tracking at different target frequencies (0 25, 0.33, 0 50, 1 Hz). The mean values are represented for the control group (with standard deviation) and for each individual of the patient groups Negative bars indicate hypometria of rightwards saccades.

[direction factor effect: F(1,225) = 2.2, $P = 0.13$]. This result was confirmed by computing a t test on the difference of the mean amplitude between right and left saccades in each patient group (RPD: $t = 1.2$, $P = 0.28$; LPD: $t = -0.11$, $P = 0.92$).

The amplitude of the non-anticipatory saccades during predictive tracking was slightly decreased only in the LPD patients [group effect: F(2,221) = 9.5, $P = 0.0001$]. The non-anticipatory saccade amplitude was independent of the stimulus frequency (Fig. 8). Both ANOVA and a t test showed significant asymmetry in the amplitude of non-anticipatory saccades due to a decrease in the amplitude for rightward saccades in patients groups (RPD, $t = -3.6$, $P = 0.02$; LPD, $t = -16$, $P = 0.00008$; Student's t test) (Fig. 8).

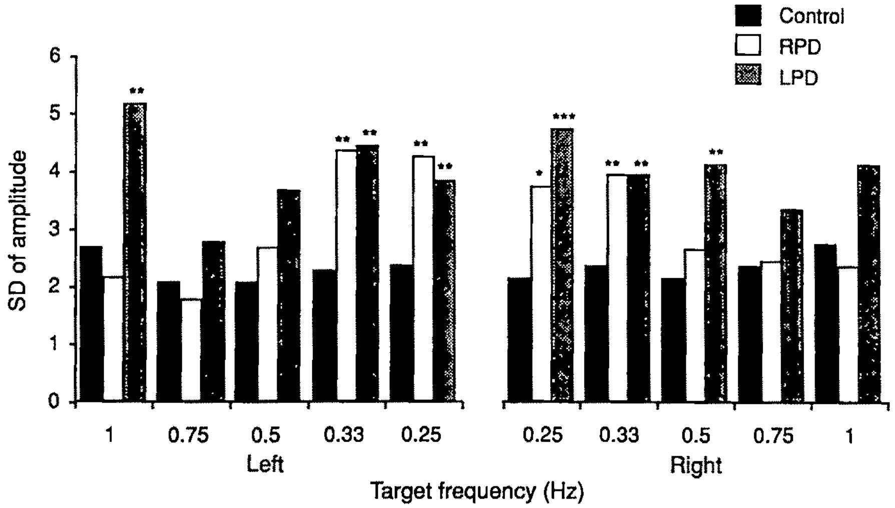

FIG. 6. Standard deviation (SD) of saccade amplitude during predictive tracking at the different target frequencies for the control, RPD and LPD groups. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Student's t test).

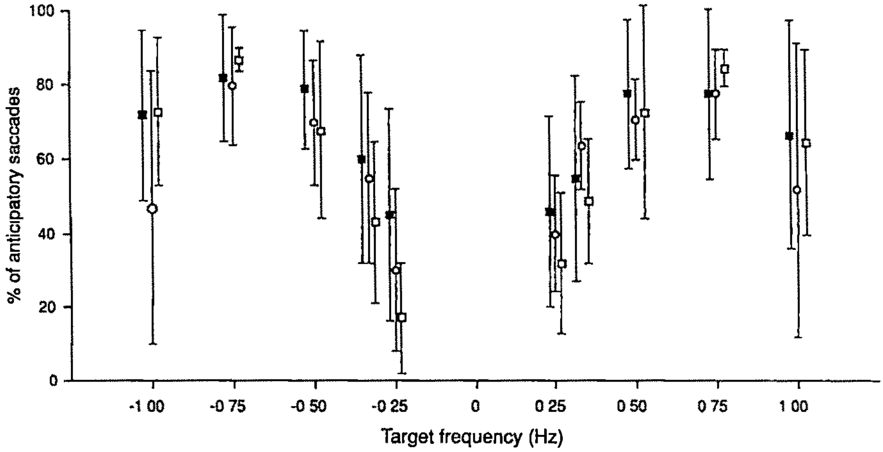

FIG. 7. Percentage of anticipatory saccades as a function of the target frequency, for the control (■), RPD (○) and LPD (□) groups. Each point represents the mean of the percentage values of all the subjects in each group.

To summarize: the decrease of saccade amplitude was mainly apparent in the anticipatory responses in both hemi-PD groups; this amplitude defect depended upon frequency and was more marked in LPD patients, but it did not correlate with response direction.

Latency-amplitude correlation

By a linear regression analysis, we found a significant positive correlation between saccade latency and amplitude at low frequencies, in both patient groups ($P < 0.0001$

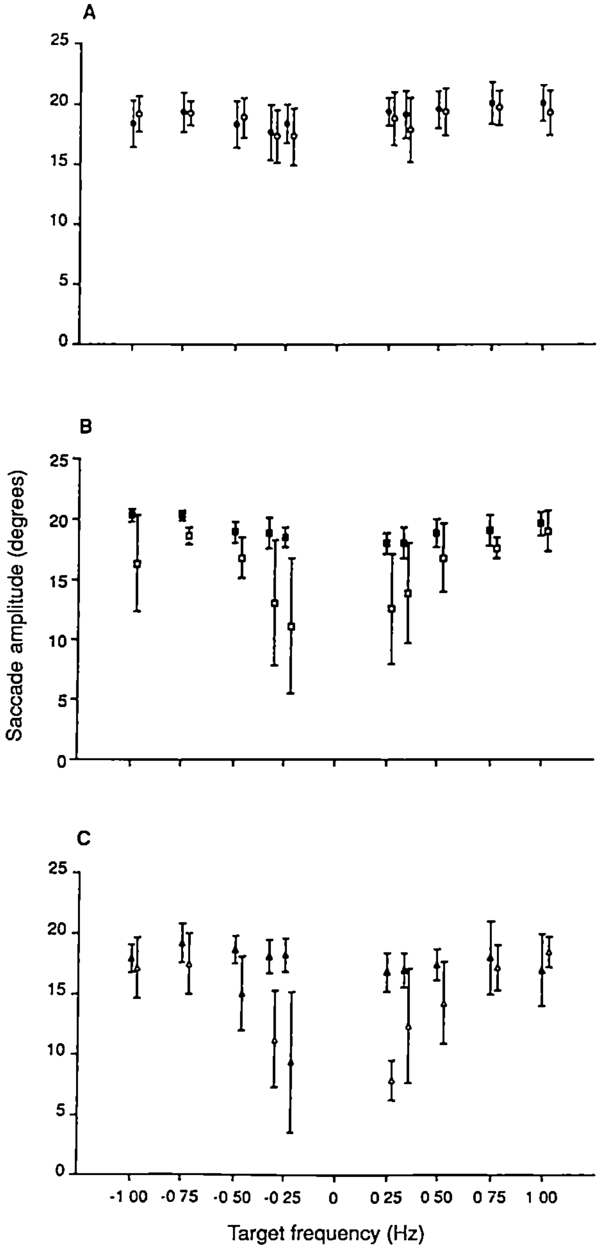

FIG. 8. Mean amplitude of the non-anticipatory (filled symbols) and of the anticipatory (open symbols) saccade as a function of the target frequency. A, the control group; B, the RPD group; and C, the LPD group.

at 0.33 and 0.25 Hz, $P < 0.05$ at 0.5 Hz) but not in controls. At high frequencies (1 Hz), a significant latency-amplitude correlation was found mainly for leftwards saccades in both patient groups.

DISCUSSION

The main finding of this study is that patients with mild hemi-PD show abnormalities of saccades during tracking of targets jumping with predictable timing and location, but not to targets jumping to random locations at unpredictable times. This effect was independent of the side affected. The patients demonstrated hypometria of saccades and increased variability in the pattern of tracking. The deficits, usually greatest at low frequencies of target motion, were largely bilateral in both RPD and LPD. However, in patients with LPD, overall saccadic hypometria (for saccades in both directions) was greater and saccade latency was asymmetric (left latency greater than right latency). On the other hand, in patients with RPD, the pattern of timing during prediction was more variable.

We will discuss these results first by reviewing some aspects of the normal physiology of the basal ganglia relevant to saccadic eye movements. Secondly, we will consider the nature of the deficits of prediction in PD. Finally, we will speculate upon the relationship between the ocular motor findings and the asymmetry of the somato-motor deficits in our patients.

Saccade abnormalities in basal ganglia disease

Normal ocular motor physiology of the basal ganglia. The superior colliculus (SC) appears to participate in the generation of all types of saccades. The basal ganglia may gate activity in the SC, to facilitate more volitional types of saccades and to suppress, when necessary, more reflexive types of saccades. By virtue of successive inhibitory pathways from the caudate nucleus (CN), to the substantia nigra pars reticulata (SNPR), and then from the SNPR to the SC, the basal ganglia can modulate activity within the SC (Hikosaka and Wurtz, 1983*a,b,c,d,* 1985*a,b*; Hikosaka *et al.*, 1989*a,b,c*). The frontal and parietal lobes may also modulate activity in the SC by direct projections as well as through projections to the CN (Petras, 1971; Fries 1984, 1985; Komatsu and Suzuki, 1985; Lynch *et al.*, 1985; Selemon and Golman-Rakic, 1985; Huerta *et al.*, 1986; Updyke, 1986; Segraves and Goldberg, 1987; Selemon and Goldman-Rakic, 1988; Stanton *et al.*, 1988).

Pathophysiology of basal ganglia disturbances of eye movements. In HD, there are pathological changes in the CN and probably also in the SNPR (Albin *et al.*, 1989; Oyanagi *et al.*, 1989). Patients with HD show defects in initiating more voluntary saccades (including those in predictive tracking paradigms) and in suppressing, uncalled-for, reflexive saccades (Lasker *et al.*, 1987; 1988; Tian *et al.*, 1991).

In PD, the main pathologic effect is seen in the SNPC. Although the SNPC has no known ocular motor function, it could influence activity in the CN, or even the SNPR, by virtue of its direct projections to both structures (Hikosaka and Wurtz, 1989). Like patients with HD, patients with PD have difficulty initiating voluntary saccades (Bronstein and Kennard, 1985; Crawford *et al.*, 1989*a*; Lueck *et al.*, 1990).

However, the saccadic eye-movement defects in PD differ in some respects from those in HD. A loss in the ability to suppress uncalled-for reflexive saccades, the oculomotor *sine qua non* for HD (Lasker *et al.*, 1987), has not been observed in PD (Crawford *et al.*, 1989*b*). Although patients with either PD or HD have difficulty generating predictive saccades, abnormalities of latencies are more prominent in HD and abnormalities of accuracy more prominent in PD (Crawford *et al.*, 1989*b*; Tian *et al.*, 1991).

The reasons for the differences in eye movements between HD and PD are not clear. Dysmetric and delayed saccades are produced by acute pharmacological lesions of the SC (Hikosaka and Wurtz, 1985*a*, 1986; Lee *et al.*, 1988). It is possible that both the level of tonic activity in the SNPR, and the level of phasic activity that occurs in relation to saccades (either in the CN or in the SNPR), determine whether or not there will be hypometria and/or delay in initiating saccades. Which abnormality predominates in patients with HD or PD might depend upon the relative differences between alterations in phasic and in tonic activity emanating from the basal ganglia.

On the other hand, it is difficult to attribute specific ocular motor defects to malfunction in a single structure or in a particular population of neurons, since defects in one structure often lead to changes in distant ones. For instance, in PD, metabolic changes affect not only the substantia nigra and striatum but also the thalamus and the frontal lobes (Wolfson *et al.*, 1985; Ho *et al.*, 1988; Mitchell *et al.*, 1989; Palombo *et al.*, 1990).

Predictive tracking in normal subjects and in patients

Accurate predictive saccadic tracking requires that the temporal (timing) and the spatial (location) components of the motion of the target are correctly stored in memory and then used, in lieu of direct visual information, to trigger a saccade of the right amplitude at the right time. In normal subjects, the ability to anticipate the motion of the target in a predictive tracking task is best at a target frequency of about 0.5 Hz. At lower and higher frequencies the degree of anticipation is less though accuracy is relatively maintained.

Only a few studies have reported the effects of focal structural lesion on predictive tracking capabilities. Lesions of both frontal and parietal structures have been implicated in predictive tracking deficits but specific details of how the lesions affected amplitude versus timing are scanty (Pyykkö *et al.*, 1984; Bruce and Borden, 1986; Sharpe, 1986; MacAvoy and Bruce, 1989; Ron *et al.*, 1989). In patients with HD, the predominant defect in predictive tracking is an increase in latency; the degree of anticipation is considerably less than that of normal subjects (Tian *et al.*, 1991).

Abnormalities of predictive tracking in PD

Patients with PD also show abnormalities during predictive tracking but the degree of defect is subject to the specific nature of the instructions and of the task (Bronstein and Kennard, 1985; Crawford *et al.*, 1989*b*). Performance was relatively worse if the patient was informed of the predictive nature of the task and relatively better when an auditory cue was substituted for a visual one.

Amplitude disorders. The most striking defect of prediction in PD is hypometria of saccades (Bronstein and Kennard, 1985; Crawford *et al.*, 1989*b*). Our patients, too, showed a decrease in amplitude of predictive saccades and we also found that the degree of hypometria was closely related to the latency; saccades made at lower latencies were smaller. Moreover, the amplitude of saccades during a period of tracking was more variable than in normal subjects. During predictive tracking, as expected, hypometria was present mainly for the anticipatory saccades (latency < 100 ms) since hypometria was not present for saccades made in the reflexive paradigm. Presumably during the predictive paradigm, all saccades made within 100 ms of the target jump had no immediate visual information available to assist in programming saccade amplitude while saccades made well after the target jumped (e.g. >250 ms) had visual information available to adjust saccade size. Non-anticipatory saccades made with latencies between these extremes probably reflect a mixture of internally generated and visually guided movements. Hence, they would be expected to, and did, show intermediate values of saccade amplitude.

The explanation for the hypometria of anticipatory saccades in PD does not appear to be one of an inaccurate memory of the spatial location of the target, since patients with PD have been reported to make corrective saccades to the actual target location, even without new visual information (Crawford *et al.*, 1989*b*). Thus, during predictive tracking in PD, the problem is not an inability to register accurately the location of the targets in memory; rather it is an inability to generate an initial saccade of a large enough amplitude.

Timing disorders. The abnormality of predictive timing in our patients was not simply a global decrease in the ability to anticipate the position of the target; the mean latencies of the patient groups were not significantly different from those of normal subjects. Rather, the pattern of tracking was less consistent, as reflected in more fluctuation of the mean values of latency throughout each period of tracking at a given stimulus frequency. Whether this defect is part of a generalized loss of fidelity of performance, as is suggested from the increase in the overall variability of saccade latency, or reflects a specific defect in the ability to store and to use timing information during predictive tracking, is not known.

Frequency dependency of predictive deficits. In our patients, the defects in timing and amplitude were more prominent at lower frequencies. Occasionally, defects were also seen at higher frequencies. Furthermore, the effect of frequency, *per se*, was greater in patients than in normal subjects. At low frequencies, prediction may be more impaired because of an inability to sustain the memory trace of target timing, while at 1 Hz, PD patients may falter in prediction in a similar way to that described by Dejong and Jones (1971) for eye movements and by Flowers (1978) and Bloxham *et al.* (1984) for manual tracking. Whether these effects of frequency are specific to patients with PD or also occur in patients with other conditions in which predictive behaviour is impaired, remains to be determined.

Asymmetrical ocular motor function in hemi-PD

Directional asymmetry in hemi-PD patients. Why did our patients with hemi-PD not show more directional asymmetry of their saccades abnormalities, considering the remarkable asymmetry of their somato-motor syndrome? Prior studies in hemi-PD have shown minimal or no directional asymmetries in saccades to randomly appearing targets (Carl and Wurtz, 1985; Rascol *et al.*, 1989). Only in memory-guided saccades has a clear-cut asymmetry, in peak velocity (slower to the side of the somato-motor deficit) but not in amplitude or latency, been reported.

In interpreting results in hemi-PD one must keep in mind several caveats. First, it may be that the ocular motor abnormalities correlate with only one aspect of the somato-motor syndrome, for example the rigidity, the bradykinesia or the tremor. Our patients were heterogeneous with respect to somato-motor abnormalities though there was no obvious correlation between the predominant somato-motor finding and the ocular motor deficit. Secondly, it had been shown with positron emission tomography in patients with hemi-PD that asymmetrical activity in the putamen was correlated with the somato-motor syndrome, while the decrease of activity with the CN was not (Leenders *et al.*, 1990). Since the latter structure is more likely to be related to the control of eye movements, a large asymmetry in saccade performance might not be expected. Thirdly, predictive tracking in either direction may be behaviour that can be relatively well elaborated by either cerebral hemisphere. With respect to this last point there are bilateral cortical-striatal projections and considerable interhemispheric connections between the frontal lobes (Selemon and Goldman-Rakic, 1985, 1988; Stanton *et al.*, 1988). These anatomical interconnections might account for the largely bidirectional nature of the ocular motor deficits in prediction in hemi-PD.

Directional asymmetries in experimental hemi-PD. In contrast, experimental studies in monkeys made acutely hemi-parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, have shown marked asymmetry of saccade function. Saccades were delayed, hypometric and slowed when directed toward the side of the affected limb (Miyashita *et al.*, 1990; Usui *et al.*, 1990). These deficits were greatest for memory-guided saccades; predictive capabilities were not examined. Furthermore, these experimental studies are not directly comparable to our results since, in patients there is often subtle involvement of the non-affected side of the body even when the somato-motor syndrome is predominantly unilateral. Likewise, since the pace of damage in human subjects with hemi-PD is much slower, more compensation would be possible than in the relatively acute animal models of hemi-PD.

Cerebral hemispheral specialization and PD

There is some evidence that the right and left cerebral hemispheres contribute to overall motor control in slightly different ways. The right hemisphere is thought to be more involved in the integration for spatial localization of the position of the target and the left hemisphere in programming and learning bilateral motor sequencing (Kimura and Archibald, 1974; Jason, 1983*a*,*b*; Haaland *et al.*, 1987; Fisk and Goodale, 1988; Harrington and Haaland, 1991). Although the ocular motor deficits in our patients were largely bidirectional, our patients with hemi-PD did show several side-to-side differences in predictive tracking.

For LPD patients, predictive latencies were greater for leftward saccades. This finding is consonant with the idea that the right cerebrum, including the basal ganglia, may be relatively more important for triggering leftward than rightward saccades, whereas the left cerebral hemisphere may be relatively equi-potent for triggering saccades in either direction. Perhaps related is the finding that hemi-neglect in human patients is usually more prominent with right cerebral lesions. Furthermore, neuropsychological tests have shown a mild left hemispatial neglect in patients with LPD (Starkstein, 1987; Levin *et al.*, 1991). This asymmetric defect in attention may account for the asymmetry in predictive latency in our patients with LPD. It is interesting that there was no asymmetry in latency for more reflexive, visually guided saccades, which suggests the idea that our patients could have neglect that is specific for internally generated, rather than for externally triggering behaviour.

Our patients with LPD also showed a greater degree of overall hypometria, and more variability, than did RPD. Whether or not these findings reflect a specific aspect of right cerebral hemisphere function related to programming the spatial components of predictive tracking remains to be proven.

On the other hand, patients with RPD showed more variability of saccade latency, across a wider range of frequencies, than did LPD patients. This finding suggests that the left cerebral hemisphere may play a more critical role in generating a precise estimate of the timing of the target during prediction.

To summarize, our results suggest that the left and the right cerebral hemispheres make slightly different contributions to the timing and the spatial components that comprise optimal predictive saccadic behaviour. Furthermore, this finding is compatible with the idea that the functions of the basal ganglia are intimately related to cerebral cortex via reciprocal loops (Alexander *et al.*, 1986; Alexander and Crutcher, 1990). Further studies in patients with focal cerebral (and basal ganglia) lesions may confirm or refute these speculations about hemispheric specialization in predictive ocular motor behaviour.

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RELATIONSHIP BETWEEN ELECTROMYOGRAPHIC ACTIVITY AND CLINICALLY ASSESSED RIGIDITY STUDIED AT THE WRIST JOINT IN PARKINSON'S DISEASE

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SUMMARY

The electromyographic (EMG) patterns recorded from wrist muscles during manually applied, repetitive flexion and extension movements of the wrist joint, used for simultaneous clinical assessment of rigidity, were studied in patients with Parkinson's disease and healthy subjects. Recordings were made whilst patients/subjects attempted voluntarily to relax the muscle of the arm whose wrist joint was manipulated. Individual patients were investigated before and at varying times after their routine daily medication as their clinical rigidity underwent associated modulations. It was often possible to induce additional alterations in clinical rigidity by instructing patients to perform an activation or Jendrassik-like manoeuvre (clenching the contralateral fist). In rigid patients, the approximately sinusoidal wrist displacements (60 deg, 1–1.5 Hz) typically elicited pronounced, cyclic modulations of EMG activities in wrist flexors and extensors; increases in EMG activity were phase-locked to the respective periods of muscle stretch. Stretch-related EMG activity reduced or disappeared as rigidity was abolished by drug therapy. The EMG patterns of patients showing cogwheel rigidity featured discrete, phasic bursts superimposed upon more generalized stretch-related increases in activity. In healthy subjects, showing no clinical rigidity, the pronounced cyclic modulations of EMG activity characteristic of rigid patients were absent during similar manually applied wrist displacements. Quantitative EMG measurements for individual patients, made 'on' and 'off' medication and as their rigidity fluctuated, indicated that mild (grade 1) and moderate (grade 2) rigidity was consistently associated with increased stretch-related activity compared with non-rigid conditions. Pair-wise statistical analysis indicated such increases in EMG to be significant. Similarly, the ratios of EMG activities in the stretched versus released muscles were significantly greater for grades 1 and 2 rigidity than in the absence of rigidity. Overall, the present findings support the view that enhancement of stretch reflex activity has a major role in the genesis of parkinsonian rigidity.

INTRODUCTION

The pathophysiology of rigidity in Parkinson's disease remains obscure. Two main types of explanation have been advanced. First, that parkinsonian rigidity is of reflex origin (Pollock and Davis, 1930); secondly, that changes in the intrinsic mechanical properties of muscle are mainly responsible (Dietz *et al.*, 1981).

The former hypothesis is supported by the classical findings that rigidity is abolished following both dorsal root section (Foerster, 1921) and the intramuscular injection of local anaesthetic (Walshe, 1924); taken together, these observations suggest that activation

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of reflex pathways by afferent input from muscle receptors is essential for the expression of rigidity. Although the H-reflex, tendon jerk and 'short-latency' stretch reflex pathways appear to operate normally (Angel and Hofmann, 1963; Dietrichson, 1971; Tatton and Lee, 1975, but *see* Noth *et al.*, 1988; Rack, 1990), it is generally agreed that 'long-latency' stretch reflex activity is enhanced in Parkinson's disease (Tatton and Lee, 1975). However, despite early reports of a quantitative association between long-latency stretch responses and rigidity (Lee and Tatton, 1975; Mortimer and Webster, 1979), numerous recent investigations reappraising this issue have failed to confirm such a clear-cut relationship (Berardelli *et al.*, 1983; Rothwell *et al.*, 1983; Cody *et al.*, 1986; Meara and Cody, 1989). Thus, the present concensus is that whilst exaggerated stretch reflex activity plays a part in the generation of parkinsonian rigidity the importance of its contribution is uncertain.

It should be recognized, however, that the precise experimental conditions employed in these laboratory studies may have differed in crucial respects from those used in standard neurological examination. The forcible displacements of the joint being investigated, usually driven by a motor or comparable mechanical actuator, were often linear and quite unlike the rhythmic, approximately sinusoidal movements applied manually by a clinical examiner; additionally, patients were often required to produce a steady background contraction rather than to attempt to relax. Thus, disparities in the balance of excitation of the various classes of sensory receptors and the prevailing 'set' of the central neural pathways are to be expected. These could lead to the essential reflex mechanisms operating during clinical testing remaining undetected.

The present experiments were undertaken to address this problem by the novel but straightforward approach of recording electromyographic (EMG) activities and displacement during actual clinical testing of rigidity at the wrist and relating these to the rigidity simultaneously appreciated by the examiner. Furthermore, individual patients were studied before and at varying times after their rigidity was reduced or abolished by routine medication. In this way, any association between muscle activity and rigidity could be more reliably identified without recourse to pooling unpaired data where inter-subject variability might obscure an underlying relationship.

The present findings provide strong evidence that stretch reflexes of wrist muscles are enhanced in Parkinson's disease and that increased reflex activation of these muscles is an important contributor to the genesis of clinically assessed rigidity at this joint.

METHODS

Subjects

Nine patients with Parkinson's disease, of mean age 60±5 (SD) yrs, and 10 healthy subjects, of mean age 62±6 yrs, were studied. The clinical details of the patients, together with their medication, are given in Table 1. All patients had a clear response to levodopa therapy; each was studied when 'off' and 'on' therapy. In two patients clinical rigidity, unlike bradykinesia, was not reduced by medication.

Experimental arrangement

Application of wrist displacement. The subject sat in a chair with the forearm pronated and pointing forwards. The pronated arm was abducted at the shoulder and the elbow was flexed to an angle of 90 deg. Pillows on a horizontal platform supported the elbow and forearm at a comfortable height below the shoulder; the hand protruded unsupported into space beyond the platform. The examiner (R.J.M.) used one hand to apply forcible extension and flexion movements, identical to those routinely performed

TABLE 1 CLINICAL DETAILS OF PATIENTS

Patients	Age (yrs)	Duration (yrs)	Webster rating	*Off medication*			*On medication*			Drugs
				Rigidity	*Bradykinesia*	*Tremor*	*Rigidity*	*Bradykinesia*	*Tremor*	
G.T	64	8	22	1	+++	0	0	+	0	S 110 qds S 275 1 or 2 bd O 50 mg tds
G.W	50	7	16	0.5	+++	(+)	0.5	+	0	S 110 tds O 50 mg tds
P M.	64	8	13	2	++	0	0	+	0	M 250 tds P 5 mg tds
J.B.	68	11	20	1	++	(+)	0	+	0	S 110 qds B 5 mg tds
J.A.	55	9	18	1	++	0	0	0	0	S 110 one daily S 110 1 or 2 tablets tds Br 10 mg tds O 50 mg tds
F.P.	64	5	17	1	++	0	0	0	0	SP six times Se 20 mg daily
L.J	71	11	20	1	+++	(+)	0 5	+	0	S110 six times B 5 mg tds
T E.	61	16	19	1	++	0	0	+	0	S 275 1 or 2 tablets qds B 5 mg tds Se 10 mg daily
D.B.	66	5	14	1	++	0	0	0	0	SP qds Se 10 mg daily

Rigidity, bradykinesia and tremor were assessed when patients were 'off' and 'on' medication. Rigidity gradients (on five-point scale, *see* Methods) are for the wrist and were obtained under resting conditions. The values listed refer to the greatest degree of rigidity during flexion or extension. Bradykinesia and tremor were graded on a five-point scale 0 = absent, (+) = intermittent; + = mild; ++ = moderate; +++ = severe. Drugs: S = Sinemet 110/275; SP = Sinemet plus; M = Madopar 250, Se = Selegiline; Br = Bromocriptine; B = Benzhexol; O = Orphenadrine, P = Procyclidine. Abbreviations for doses: bd = twice daily, tds = three times daily; qds = four times daily.

for clinical grading of rigidity, to the subject's wrist whilst holding the subject's lower forearm with the other hand to restrict movements to that joint. During this procedure, the subject was instructed to attempt to completely relax the muscles of the arm being tested. A small accelerometer (Bruel and Kjaer, type 4332), mounted on a lightweight plate, was strapped to the dorsum of the subject's hand and orientated with its sensitive axis in the plane of extension-flexion. The accelerometer was linked to a charge amplifier (Bruel and Kjaer, type 2635). Electronic circuits within the charge amplifier performed a double integration of the transducer output and thereby converted it to a displacement signal. An inherent characteristic of the accelerometer-charge amplifier system was that for low frequencies of wrist displacement it introduced some phase non-linearities. Such phase shift errors were determined by attaching the accelerometer to the moving arm of a large electromagnetic vibrator and comparing the displacement signal of the charge amplifier with that of a precision length transducer incorporated in the vibrator. Calibrations were made for a range of frequencies (0.5 – 2 Hz) of sinusoidal movements, spanning those used during manual wrist displacement (*see below*), of the vibrator and corresponding phase errors of the charge amplifier signal were measured. These were used to make phase corrections to the accelerometer-charge amplifier signal generated during manually applied wrist movements. In addition, in separate preliminary experiments, a video camera was used to record, against an angular scale, the size and speed of passively applied movements of the wrist joint that were employed in routine clinical evaluation of rigidity. Frame-by-frame analysis of the tape indicated that approximately sinusoidal displacements of around 60 deg amplitude (from end-extension to end-flexion) were typically performed at frequencies of 1 – 1.5 Hz and with peak velocities of 120 – 200 deg/s. These movement parameters correspond closely with those previously found to elicit parkinsonian rigidity most reliably and reproducibly at the wrist (Teräväinen *et al.*, 1989). Patients exhibiting severe rigidity could not be successfully studied. In such individuals, clinical manipulation of the wrist resulted in low amplitude, poorly reproducible joint excursions due to the marked resistance encountered during passive displacement

Rigidity assessment. The examiner evaluated rigidity of the subject's wrist as the resistance which he felt as he forcibly extended and flexed the joint. Rigidity was graded according to a five-point scale: 0 = no rigidity; 0.5 = minimal and intermittently present rigidity; 1 = mild rigidity; 2 = moderate rigidity; 3 = severe rigidity. A voice commentary of the rigidity status accompanying the applied movements was recorded on magnetic tape to assist subsequent analysis. Rigidity was assessed immediately before and after initial attachment of the accelerometer to the hand and EMG electrodes to the arm. The presence of the recording apparatus produced no alteration in rigidity.

Protocol. Each patient was initially seen having been 'off' medication for approximately 12 h. The wrist exhibiting the more marked rigidity was selected for study or, in the case of symmetrical involvement, the wrist of the dominant hand. Recordings were then made under two conditions: (i) the patient attempted to voluntarily relax the muscles of both upper limbs (and of other body parts as far as was consistent with a sitting posture), and (ii) the patient attempted to relax the muscles of the arm of the wrist being assessed whilst maintaining maximal synkinetic clenching of the contralateral hand. The terms 'resting rigidity' and 'activated rigidity' are used to describe rigidity measured under these respective conditions. This follows the introduction of this terminology by Webster (1960, 1966) and its subsequent widespread adoption by investigators in the field (Subczynski *et al.*, 1963; Webster, 1968; Mortimer and Webster, 1979; Barbeau *et al.*, 1981; Teräväinen *et al.*, 1989). Recordings were made for a series, typically 10 – 20, of extension-flexion cycles under each condition. Patients were then given their regular anti-parkinsonian medication. After a clear clinical improvement in rigidity had taken place, usually 30 – 60 min, both 'resting rigidity' and 'activated rigidity' were reassessed. Many patients showed some spontaneous, short-term fluctuations in rigidity under both conditions. It was, thus, often possible to obtain recordings from an individual patient for several rigidity states and with variations between rigidity-in-flexion and rigidity-in-extension.

Electromyography. Electromyographic activities were recorded with pairs of surface electrodes over the belly of flexor carpi radialis (FCR) and over the wrist extensors. Signals were amplified, bandpass filtered (20 Hz – 3 kHz) and rectified. Electromyographic signals, together with those of wrist displacement and a voice commentary of rigidity status, were recorded on magnetic tape.

Data analysis. Computer averages were made of series of 2 s segments of simultaneously recorded wrist displacement and rectified flexor and extensor EMG data. Each 2 s segment typically comprised two full extension-flexion cycles. Averaging was initiated when the displacement waveform reached a preselected trigger level. Data segments were only averaged together when a preliminary review of the continuous recordings indicated that the movement trajectories were highly repeatable and that the rigidity state remained constant throughout.

Quantitative comparisons of the movement-related EMG activities which accompanied changes in rigidity of individual patients were made from area measurements of averaged, rectified EMG records. The approximately sinusoidal, averaged displacement records were subdivided into two phases corresponding to flexion and extension. Area measurements of the rectified EMG activities of both flexor and extensor muscles, occurring during each of these two phases, were obtained by tracing the requisite portions of EMG records with a graphics tablet feeding into a separate computer. The area measurement (μV.s) obtained, over a particular flexion or extension phase, was converted into a mean EMG activity (μV) level by dividing by the duration (s) of the movement phase. In this way, measurements over movement cycles of varying duration could be compared. Since there were considerable variations in the absolute values of mean stretch-related activity levels between patients for a given rigidity grade, as may be expected, due to differences in factors such as electrode resistance, amount of subcutaneous fat and muscle bulk, a normalization procedure was adopted to allow inter-subject comparisons. In each individual patient the mean EMG activity level in the absence (grade 0) of clinical rigidity, recorded during the stretching phase of the averaged sinusoidal displacement, was taken as the standard. The corresponding mean EMG levels in the presence of each grade (0.5, 1, 2) of detectable rigidity in that individual were divided by this value to provide a series of normalized measures of stretch-related activity. Thus, the series of normalized values of activity for a particular patient provided ratios of the extents to which his/her stretch-related EMG increased for each rigidity grade over that present in non-rigid conditions.

RESULTS

Figure 1 illustrates a representative example of a major finding of the present experiments. The EMG patterns recorded from the wrist musculature of a patient with Parkinson's disease who exhibited clinical rigidity, during manually applied extension and flexion movements of the wrist joint, underwent a dramatic change following anti-parkinsonian medication and an accompanying reduction in rigidity.

Prior to medication (Fig. 1A), when grade 1 plastic rigidity was encountered during both extension and flexion displacements of the wrist, pronounced reciprocal modulations of EMG are present in the averages of flexor and extensor activity. For both muscle groups, the periods of increased EMG activity largely correspond to the respective phases of muscle stretch, as indicated by the displacement record, whilst release of the muscles is associated with relative inactivity. In contrast, the averaged EMG records of Fig. 1B, obtained following medication and when rigidity had been abolished, show activities which are both reduced in absolute amplitudes and lacking the prominent reciprocal modulations evident earlier. The only signs of movement-related EMG are small, transient bursts in the extensors which now occur during release of the muscle and may represent delayed 'shortening reactions' (Angel, 1983).

The timing of the vigorous EMG bursts present before medication is consistent with their larely representing stretch reflex activity. Additionally, it is highly probable that these periods of muscle activation must have generated forces which opposed the wrist displacements being simultaneously applied by the examiner and, thus, have contributed to the rigidity appreciated.

Further support for this latter proposal was provided by observations in non-rigid, healthy subjects.

As illustrated in Fig. 2, EMG averages from the wrist musculature of healthy subjects showed comparatively little movement-related activity. The records of the subject featured in Fig. 2A show no clear-cut phasic bursts of discharge in either flexor or extensor muscles, such were noted for the rigid parkinsonian (Fig. 1A); the only hint of any modulation of EMG is in the extensor where activity appears to show a low-level increase

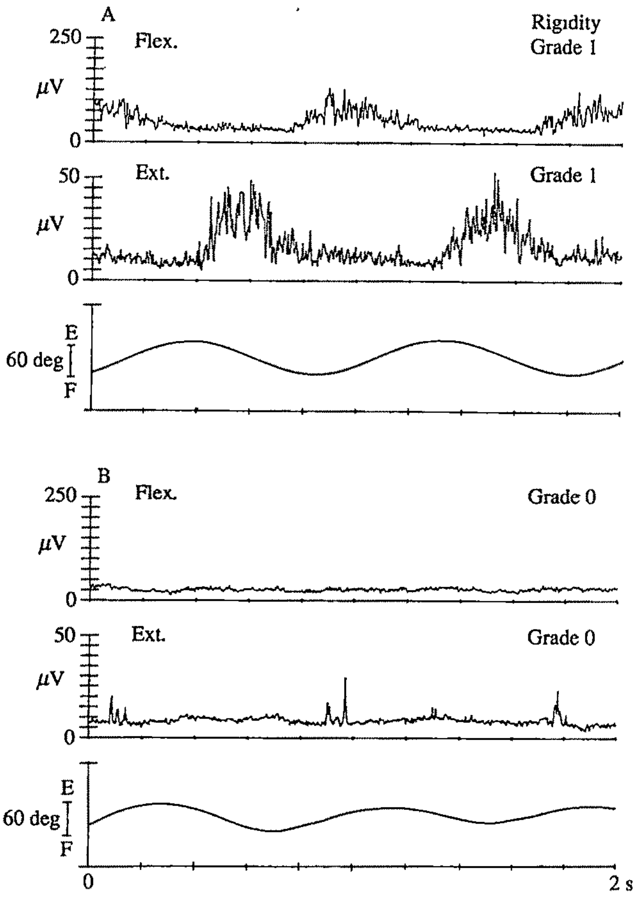

FIG. 1. Rectified, surface electromyograms from the wrist flexor (flex.) and extensor (ext.) muscles during manually applied, alternating extension (E) and flexion (F) movements of the wrist in a patient (D.B.) with Parkinson's disease. Electromyographical and displacement records are simultaneous averages from three movement sequences. The rigidity grades assessed, by the clinical examiner, during the same movements are indicated to the right. The value against the flexor EMG record denotes rigidity during extension movements and that against the extensor EMG record denotes rigidity during flexion movements. A, records obtained prior to medication. B, records obtained following medication.

late in the stretching phase which continues well into release. A generally similar pattern, from a different subject, is presented in Fig. 2B, although now a more definite modulation of extensor EMG is evident. These results in non-rigid, healthy subjects broadly resemble those previously described for parkinsonian patients following medication and abolition of their rigidity. In healthy subjects and non-rigid patients, movement-related EMG activity was either negligible and unlikely to have led to appreciable contractile force or occurred at a time when the resulting mechanical effects probably assisted movement and, thus, contributed to 'negative rigidity'.

The patient whose records are illustrated in Fig. 3 showed several interesting new features. Both the type and severity of clinical rigidity altered between activated (clenching

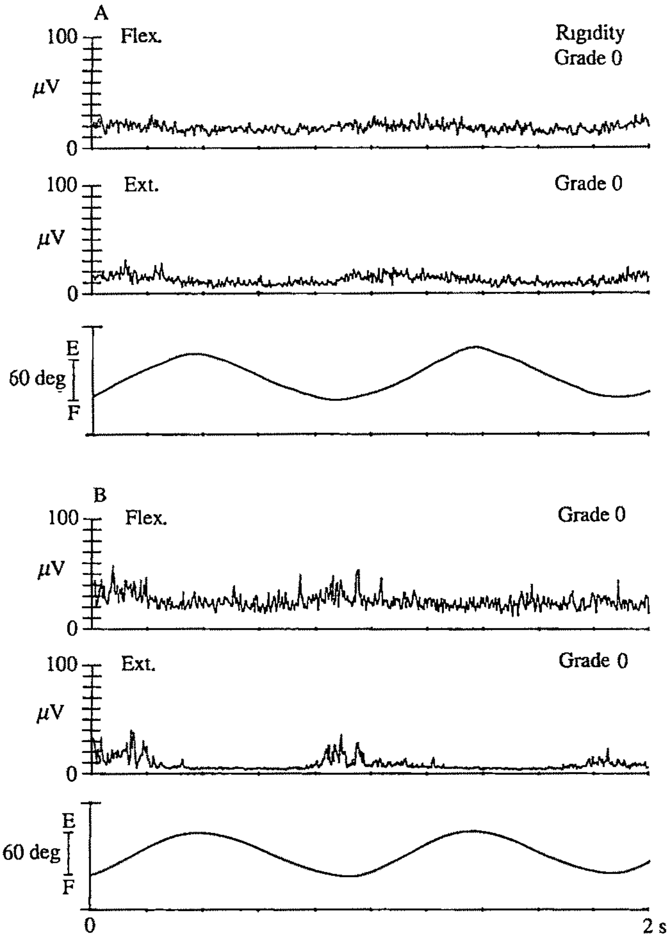

FIG. 2. Averaged (three trials) rectified, surface electromyograms and wrist joint displacement records from two healthy subjects (A, subject B.N.; B, subject B.J.).

contralateral fist, *see* Methods) and resting conditions. In addition, an asymmetrical pattern of rigidity was encountered between extension and flexion movements.

The EMG averages of Fig. 3A, obtained under activation conditions and 'off' medication, show very pronounced stretch-related increases in flexor discharge but no clear modulation of extensor activity. Simultaneous rigidity gradings were 'moderate, plastic' for extension displacements and 'absent' for flexion displacements. Figure 3B shows that upon ceasing activation procedures, the stretch-related flexor activity declined somewhat in amplitude and became segmented into fairly discrete bursts (particularly clear during the second cycle of extension). The accompanying rigidity was now assessed as 'mild, cogwheel'. Following medication (Fig. 3c) modulation of flexor EMG was almost completely suppressed; rigidity now disappeared. These findings support three features of the relationships between patterns of muscle activation and accompanying

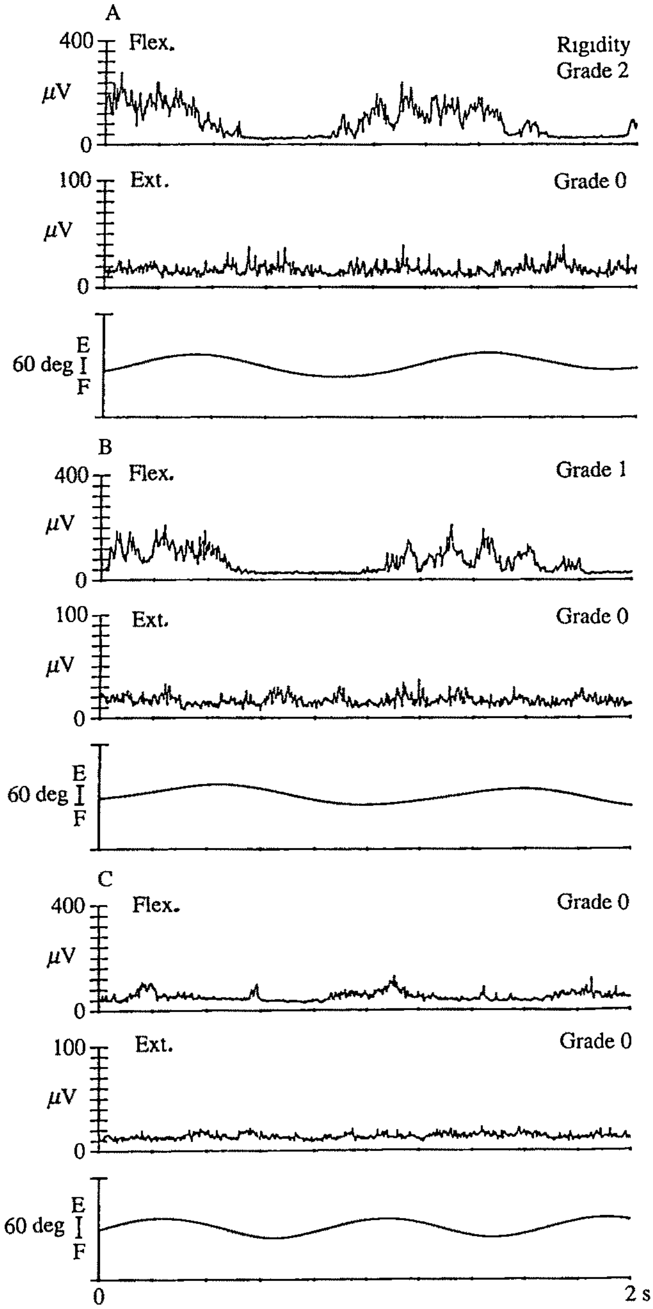

FIG. 3. Averaged (three trials), rectified, surface electromyograms and wrist joint displacement records from a patient (F.P.) with Parkinson's disease The records of A and B were obtained prior to medication; those of C were obtained following medication. 'Off' treatment the patient showed an asymmetrical pattern of rigidity during extension and flexion movements. A, grade 2 plastic rigidity was present during extension movements under activation conditions (clenching contralateral fist), whilst no rigidity was felt during flexion movements. B, grade 1 cogwheel rigidity was present under resting conditions whilst no rigidity was felt during flexion movements. 'On' treatment (C) no rigidity was felt during either extension or flexion movements.

rigidity. First, there is an association between the overall degree of rigidity and the magnitude of simultaneously evoked EMG in the stretched muscle. Secondly, that appreciable rigidity may occur in the absence of any reciprocal reduction in EMG in the released muscle. Thirdly, that shorter-term oscillations in resistance (cogwheeling) are accompanied by corresponding well-defined fluctuations in EMG within broader periods of increased, stretch-related activity.

Area measurements from rectified EMG averages were made to determine whether the changes in rigidity of individual patients, which occurred following medication, varied quantitatively with their EMG patterns.

Figure 4 compares the amplitudes of EMG activities, measured throughout phases of muscle stretch, for different rigidity grades in six patients. It shows clear responses to medication. In each individual, EMGs have been normalized to the value measured for zero rigidity (*see* Methods); in this way difficulties of interpretation arising from inter-subject variations in absolute EMG amplitudes were avoided.

In all individual instances, mild (grade 1) and moderate (grade 2) rigidity was associated with marked increases in stretch-related EMG compared with non-rigid conditions. This applied for rigidity in both extension and flexion. Paired statistical analysis indicated

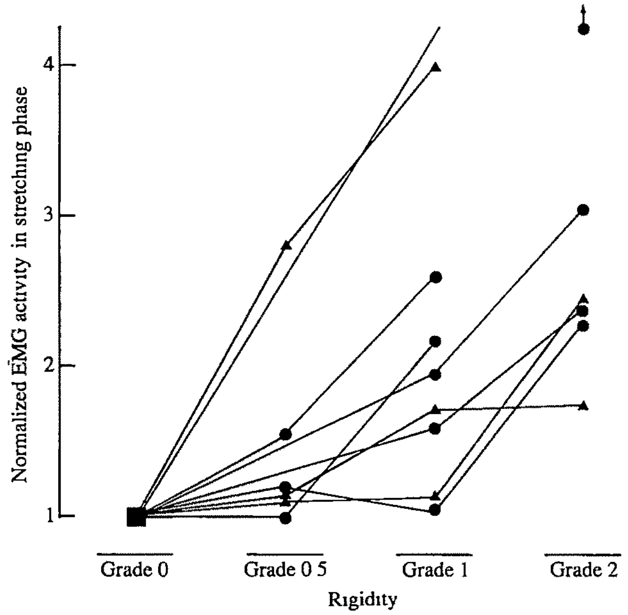

FIG 4. Relationship between normalized EMG activity in the stretched muscle and rigidity grade. Data were obtained in six patients whose rigidity changed following medication. Normalized EMG was calculated by dividing the area of the rectified, electromyographic record measured, during the stretching phase of movement, in the presence of rigidity by the corresponding value measured in the absence of rigidity (*see* Methods) Closed circles indicate measurements from flexor EMGs for rigidity during applied extension movements; closed triangles indicate measurements from extensor EMGs for rigidity during applied flexion movements. The lines join sets of data points obtained in the same patient Some patients contribute two sets of data, i.e rigidity during both extension and flexion changed after medication Rigidity grades include measurements under activation and resting conditions.

that, for this patient group, stretch-related EMG for grades 1 and 2 rigidity was significantly greater than for grade 0 (Wilcoxon, $P = 0.025$). Additionally, the stretch-related EMG accompanying grade 2 rigidity was found to be significantly ($P = 0.025$) greater than that during grade 1 rigidity. As noted in the Methods, patients with severe (grade 3) rigidity had to be excluded from the study; the examiner found it impossible to manually apply displacements of the wrist joint of comparable rate and range due to the considerable resistance encountered.

The position for minimal, intermittent (grade 0.5) rigidity was, not surprisingly, less definite. Whilst in the majority (five of six) of instances, the stretch-related EMG was increased over that in the non-rigid state, such increases were often small and paired analysis of group data showed the changes to be non-significant. Similarly, no significant differences in normalized stretch-related EMG were found between 0.5 and 1 rigidity grades.

Pair-wise statistical comparison of the absolute values of mean stretch-related EMG activities shown by individual patients for the different rigidity grades produced similar results. The absolute mean EMG levels for grades 1 and 2 rigidity were significantly higher than those for grade 0 whilst those for grade 2 were greater than those for grade 1 (Wilcoxon, $P = 0.05$). Again, no significant differences in absolute mean stretch-related EMGs were found between grades 0 and 0.5 or between grades 0.5 and 1.

[Further analysis was carried out upon the ratios of EMG activities present in the stretched muscle and the simultaneously released muscle during rigidity assessment. For example, during testing of rigidity in extension the area of rectified flexor (stretched muscle) EMG occurring during the extension displacement was divided by the area of rectified extensor (released muscle) EMG occurring over the same period. Since the net muscular force about the joint will depend on the balance of agonist versus antagonist activation, such ratios potentially provide a more sensitive index than activity in the stretched muscle alone. In the event, the results were similar to those previously described for stretch-related activity. For grades 1 and 2 rigidity, the stretched/released EMG ratio measurements were significantly greater than for non-rigid conditions (Wilcoxon, $P = 0.025$), whilst no significant differences were found between 0 and 0.5 rigidity grades.]

Considering the range of rigidity grades (0, 0.5, 1, 2) encountered, a highly significant correlation existed between normalized stretch-related EMG and the degree of rigidity (Kendall, $P < 0.001$).

Overall, therefore, these quantitative analyses suggest that stretch-related EMG makes a major contribution to concurrent rigidity.

DISCUSSION

Evidence for a muscular origin of parkinsonian rigidity

The main issue addressed by the present experiments was whether the increased resistance to manually applied joint displacements in Parkinson's disease, commonly detected by clinical examiners, is attributable to enhanced activation of the patient's muscles acting at the joint. An alternative possibility, that hypertonia results from alterations in the mechanical properties of muscle fibres or connective tissue (Dietz *et al.*, 1981), was indirectly tested. In the event, the study's findings, for the wrist joint, demonstrate the occurrence of abnormal patterns of muscular activation during standard clinical evaluation of tone in parkinsonism and strongly support their making an important contribution to the generation of rigidity.

Three complementary types of observations indicate an association between rigidity and muscular activity. First, in rigid patients alternating, manually applied, extension-flexion wrist movements generated pronounced cyclic modulations of EMG activities in the wrist muscles, whereas similar movements in non-rigid patients and in healthy subjects produced little or no movement-related activity. Furthermore, in examples of asymmetrical rigidity, between extension and flexion, correspondingly marked EMG modulation occurred in only one group of antagonist muscles. The phase-relations of EMG modulations, with increased activity occurring in the stretched muscles, were consistent with any resulting contractions opposing continuing passive movements and, thereby, contributing to accompanying rigidity. Secondly, as a patient's rigidity was reduced or abolished following medication, there was a corresponding decline in stretch-related EMG. Analysis of group data indicated a correlation between the degree of rigidity and stretch-related EMG. Additionally, the ratio of EMG activities in the stretched versus released muscles decreased. Observations in cases of asymmetrical rigidity suggest that rigidity is more closely associated with increased EMG in the stretched muscles rather than a reciprocal reduction in EMG in the simultaneously released muscles. Thirdly, in instances of rapid fluctuations in resistance during a passive movement (cogwheel rigidity) the stretch-related EMG showed discrete, high-frequency bursts whilst in plastic rigidity EMG waxed and waned in a smoother manner.

The issue of whether altered patterns of muscular activation occurring during clinical testing provide the sole and causal explanation of patients' rigidity is more problematic. The precise relationship between enhancement of stretch-related EMG and rigidity grade varied between individual patients. This probably simply reflects the clinical scale being rather coarse with each grade covering a broad range of degrees of tone. Furthermore, the clinical scale is clearly non-linear with the absolute increase in resistance between grades 1 and 2 being much greater than between grades 0 and 1. All instances of moderate (grade 2) rigidity and three-quarters of cases of mild (grade 1) rigidity were accompanied by pronounced increases in stretch-related EMG over non-rigid conditions. However, in a few of these patients mild (grade 1) rigidity was detected in the absence of any definite enhancement of motor activity. Possibly this arose from uncertainties in subjective clinical grading of marginal hypertonia. Another possibility is that, in these exceptional cases, the balance of stretch reflex excitation of different muscles within the wrist flexor (or extensor) groups may have varied from the usual pattern so that, for example, flexors other than FCR were relatively more powerfully activated and generated mechanical resistance whilst contributing little to the recorded EMG signal. Alternatively, some other unknown mechanism may have operated.

Overall, whilst it seems clear that abnormal patterns of muscular activity play an important part in generating parkinsonian rigidity at the wrist it would be unreasonable to rule out other contributory factors. Conversely, it would be unjustified to uniquely attribute hypertonia to altered mechanical properties of muscle fibres, resulting from disuse, as proposed for the lower limb from studies of gait (Dietz *et al.*, 1981), to the exclusion of ongoing motor activation patterns. Whereas central neural circuits and motor activity may be expected to be rapidly influenced by anti-parkinsonian drugs it is difficult to envisage muscle fibre mechanics changing over a comparable timescale.

Neural mechanisms of enhanced stretch-related EMG in Parkinson's disease

It is highly unlikely that the increased EMG activity observed, during periods of muscle stretch, in rigid parkinsonians was voluntary in nature. Whilst it has been suggested that patients with Parkinson's disease have difficulty in voluntarily, tonically relaxing their muscles (Marsden, 1982), this did not appear to be a common feature in our group of patients. Electromyographical activity under 'relaxed' conditions, in the absence of passive manipulation of joints, did not decrease systematically following medication and reductions in clinical rigidity. Furthermore, an explanation in terms of willed motor activation would involve not only a generalized change in volitional behaviour between non-rigid and rigid conditions but deliberate cyclic increases in voluntary drive.

A more probable explanation is that the increased stretch-related EMG found in rigidity was of reflex origin. This proposal is in accord with the classical findings of the abolition of rigidity by dorsal root section (Foerster, 1921) and intramuscular injection of local anaesthetic (Walshe, 1924). Additionally, numerous investigators have reported changes in stretch reflex activity in Parkinson's disease. It is generally agreed that the long-latency components of the stretch reflex of a variety of muscles, including those of the wrist, are enhanced in parkinsonism (Tatton and Lee, 1975; Mortimer and Webster, 1979; Berardelli *et al.*, 1983; Rothwell *et al.*, 1983). Additionally, recent evidence suggests that, for wrist muscles, earlier components of stretch reflexes may also be exaggerated in this disorder (Rack, 1990).

Accepting that the stretch-related EMG recorded during clinical examination was of reflex origin, there must have been a genuine increase in reflex responsiveness in the rigid state. The absence of well-developed reflex modulation in healthy subjects is consistent with the findings of Brown *et al.* (1982) that human stretch reflexes have a rather high threshold and low gain under relaxed conditions. The enhancement in stretch reflexes in rigidity was not simply a consequence of higher background excitability; the amplitudes of pre-existing EMG levels and of periods of stretch-evoked activity did not co-vary systematically. This conclusion echoes the earlier demonstration by Rack and Ross (1986) that parkinsonians, unlike normal subjects, may show definite reflex responses to small, low frequency (e.g. 6 deg, 2–6 Hz) sinusoidal joint displacements, despite an absence of EMG prior to the movement. However, evidence of increased reflex responsiveness appears to have been a more consistent feature of the rigid patients in the present study than the patients, in whom tremor was the more prominent clinical sign, investigated by Rack and Ross (1986). This may reflect differences in the neuropathology predisposing to rigidity versus tremor. Alternatively, the more protracted stretches employed in our experiments may have favoured the generation of exaggerated reflexes in rigid patients. Lee and Tatton (1982) reported that the production of long-latency stretch reflexes in the wrist muscles of healthy subjects depended critically upon the duration (but not amplitude or velocity) of the applied perturbation. Therefore, although little can be said with certainty of the precise mode (e.g. gain, threshold) of stretch reflex enhancement in rigidity or of whether short- or long-latency reflexes were preferentially modified, it is tempting to believe that the changes included an augmentation of long-latency stretch reflex mechanisms.

Reflex responsiveness and rigidity

So far, it has been separately argued that parkinsonian rigidity arises largely from increased motor activity and that such activity results from augmented stretch reflexes. Accordingly, rigidity is considered to have a primarily reflex origin. Indeed, several investigators have proposed a quantitative association between long-latency EMG stretch responses and rigidity and a probable causal relationship (Lee and Tatton, 1975; Tatton and Lee, 1975; Mortimer and Webster, 1979). However, others have failed to confirm these findings (Berardelli *et al.*, 1983; Rothwell *et al.*, 1983). Indeed, Cody *et al.* (1986) noted considerable variability between the behaviour of different patients so that clinically similar states could be accompanied by different stretch responses and vice versa. Consequently, they concluded that if altered stretch reflexes are basically responsible for rigidity there must be some additional essential and independently regulated mechanism(s) which was absent during their measurement of reflex responsiveness but which operates during clinical testing. In this case, much of the variation between earlier reports probably arose from differences in the particular laboratory methods and conditions employed to test reflex function. Our present findings, during replication of clinical rigidity assessment procedures, support this view and emphasize the importance of the prevailing 'set' of reflex circuits in determining reflex behaviour.

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THE AUDITORY STARTLE RESPONSE IN THE STEELE-RICHARDSON-OLSZEWSKI SYNDROME AND PARKINSON'S DISEASE

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SUMMARY

The startle response to an unexpected auditory stimulus was studied in eight patients with a clinical diagnosis of the Steele-Richardson-Olszewski syndrome (SRO), 11 patients with idiopathic Parkinson's disease (PD) and 12 normal subjects. The patients with PD were studied 'on' at the time of maximal effect of their treatment; five of these patients were also studied in their 'off' state without treatment. The auditory startle response was absent in three patients with SRO: in the remaining five the latency to onset of earliest electromyography activity (EMG) of the auditory startle response was delayed and few muscles (orbicularis oculi, sternocleidomastoid and rectus abdominis) were recruited in the response. In PD the auditory startle response was similar to that recorded in normal subjects, both in terms of the pattern of muscles recruited and the amplitude of the EMG responses, but the latency of responses in orbicularis oculi and sternocleidomastoid muscles were significantly delayed. This result was not influenced by treatment with L-dopa. In patients with SRO the finding of an abnormal startle response is consistent with loss of neurons in the lower pontine reticular formation. This region is intimately involved in the startle response in animal studies. In patients with PD the late auditory startle response might be related to withdrawal of facilitatory input to brainstem centres and reticulospinal pathways from basal ganglia. The similarity of the responses in patients when 'on' and 'off' suggests these pathways are not under potent dopaminergic control.

INTRODUCTION

In previous papers we reported an electrophysiological study of the human auditory startle response in normal subjects (Brown *et al.*, 1991*a,c*) and in patients with exaggerated startle syndromes (Brown *et al.*, 1991*b*). The results suggested that the human startle response to unexpected auditory stimuli originates in the brainstem and spreads rostrally and caudally to recruit muscles innervated by the cranial nerves and spinal cord, respectively. The efferent conduction speed of the projections to the spinal cord was found to be only half that of the fastest conducting corticospinal fibres. In addition, intrinsic muscles of the hand and foot were recruited (if at all) with an excessively long delay compared with more proximal limb muscles. These observations suggest that reticulospinal pathways are involved in the response. These characteristics of the human startle response are very similar to those reported previously in extensive studies of auditory startle responses in rats and rabbits (Davis *et al.*, 1982; Hori *et al.*, 1986;

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Wu *et al.*, 1988). The ventral pontine reticular formation, in particular, the nucleus reticularis pontis caudalis was identified as one of the crucial zones in the generation of auditory startle responses in animals (Grillner *et al.*, 1968; Hammond, 1973; Groves *et al.*, 1974; Davis *et al.*, 1982). Reticulospinal pathways were thought to be the main pathway conveying excitation to the spinal cord.

In the present paper we have examined the startle response in two further groups of patients: the first group were eight patients with a clinical diagnosis of the Steele-Richardson-Olszewski syndrome (SRO); the second group comprised 11 patients with idiopathic Parkinson's disease (PD). In SRO syndrome there are widespread pathological changes in the brainstem including degeneration of the pontine reticular formation (Steele *et al.*, 1964) with severe neuronal loss (Malessa *et al.*, 1991). The auditory startle response was absent or distorted in SRO patients. These abnormalities are consistent with this area of the brain being involved in the generation of the human startle response. In contrast, the pontine reticular formation has been found to be normal in PD (Halliday *et al.*, 1990). The auditory startle response in PD was of normal configuration but delayed, suggesting an abnormality of the basal ganglia output pathways to the brainstem.

PATIENTS

The auditory startle response was recorded in 11 patients with a clinical diagnosis of idiopathic PD (10 men, one woman), aged 42–75 yrs (mean ± SD, 55 ± 10.8 yrs), 12 age-matched control subjects (mean age ± SD 51 ±20.8 yrs) and eight patients with a clinical diagnosis of the SRO syndrome aged 54–82 yrs (mean ± SD 67.2 ± 8.8 yrs). All patients and normal subjects gave informed consent for the study which was approved by the local ethical committee. The group of patients with idiopathic PD fulfilled the diagnostic criteria of Hoehn and Yahr (1967) and all exhibited a significant improvement in their motor performance in response to treatment with L-dopa and a peripheral decarboxylase inhibitor. A clinical diagnosis of the SRO syndrome was made in all cases according to the criteria of Lees (1987). The clinical characteristics of the patients and their treatment are summarized in Tables 1 and 2. Mean (±SD) duration of disease was 8.9 ± 4.7 yrs (range 3–17 yrs) for patients with PD and 4.5 ± 2.1 yrs (range 2.5–8 yrs) for patients with the SRO syndrome. All patients with PD were studied at the time of maximal effect of their usual morning dose of L-dopa (i.e when 'on'). Five of these patients were also studied before the morning dose, 16 h after their last L-dopa dose at peak disability (i.e. when 'off'). Motor disability was evaluated

TABLE 1 CLINICAL CHARACTERISTICS AND THERAPY OF THE 11 PATIENTS WITH PD

Case	*Sex*	*Age (yrs)*	*Disease duration (yrs)*	*Hoehn and Yahr grade*	*Daily L-dopa dose*	*Other treatment*
1	F	65	7	III	500	Nil
2	M	42	9	III	700	Amantadine 300 mg, artane 6 mg
3	M	64	13	IV	700	Bromocriptine 5 mg, selegiline 10 mg
4	M	54	12	III	1500	Bromocriptine 10 mg
5	M	52	14	III	750	Apomorphine 4 8 mg, selegiline 10 mg
6	M	43	3	III	450	Benzhexol 3 mg
7	M	45	5	II	200	Lisuride 0.1 mg
8	M	48	3	II	200	Lisuride 0.2 mg
9	M	66	17	III	700	Selegiline 10 mg
10	M	75	10	III	1000	Artane 4 mg, selegiline 10 mg
11	M	52	5	III	600	Bromocriptine 15 mg, selegiline 10 mg

L-dopa therapy was administered in combination with a peripheral decarboxylase inhibitor in all cases

TABLE 2 CLINICAL CHARACTERISTICS AND DAILY THERAPY OF THE EIGHT PATIENTS WITH A CLINICAL DIAGNOSIS OF SRO SYNDROME

Case	*Age*	*Sex*	*Disease duration (yrs)*	*Ophthalmo-plegia*	*Axial dystonia*	*Pseudo-bulbar palsy*	*Frontal lobe signs*	*Akinesia-rigidity*	*Postural instability*	*Treatment*
1	82	M	3	+	+	+	+	+	+	Nil
2	65	F	3	+	+	+	+	+	+	Imipramine 75 mg
3	75	F	6	+	+	+	+	+	+	Nil
4	73	F	3	+	+	+	+	+	+	Nil
5	64	F	8	+	±	+	+	+	+	Nil
6	62	F	4	+	+	+	+	+	+	Dothiepin 50 mg, L-dopa 1000 mg
7	54	M	2 5	+	±	±	+	+	+	Amitriptyline 75 mg
8	63	M	7	+	+	+	+	+	+	Amitriptyline 75 mg

All patients fulfilled the clinical criteria for the diagnosis of this syndrome (Lees, 1987) and all had signs of a supranuclear ophthalmoplegia, including downgaze.

using the motor subscale of the UPDRS scale (Fahn *et al.*, 1987) The treated score (at the time of maximum effect of L-dopa) and the basal score (at the time of maximum disability without L-dopa) were recorded. All patients with PD were dramatically responsive to L-dopa since their percentage improvement in motor disability was greater than 50% (mean basal score = 50 ± 12, treated score = 11 ± 7).

METHODS

The startle response was elicited using an auditory tone burst of 1000 Hz, 50 ms duration and 90 dB given through earphones at random at intervals of about 20 min. Three or four trials were recorded in each individual whilst they sat comfortably in a chair, with their eyes closed. A long interstimulus interval was chosen to minimize habituation of the response with repeated presentation of the stimulus (Brown *et al.*, 1991*a*). Electromyographic (EMG) recordings were made using bipolar silver-silver chloride electrodes placed 2 cm apart over the orbicularis oculi, mentalis, sternocleidomastoid, biceps, triceps, forearm flexors, forearm extensors, abductor pollicis brevis, rectus abdominis, tibialis anterior and soleus muscles. Records were collected as single sweeps and included 50 ms of background EMG activity before the auditory stimulus. The sampling rate was 1500 Hz per channel. The latency to onset of unrectified EMG activity was measured by visual inspection of single trials on a computer display. The size of the response was defined as the amount of integrated EMG activity in the 50 ms period following its onset. In each subject, the latency and amplitude of the response in each muscle for each trial in which EMG activity was detected was measured and averaged.

For comparison with the startle response, we also investigated the auditory blink reflex in four of the patients with the SRO syndrome, six of the patients with PD and six normal subjects. The auditory blink was elicited with the same auditory stimulus as for the startle, except that the stimuli were given more frequently, approximately every 20 s rather than every 20 min. This rate of stimulation evoked only a blink reflex, for the generalized startle response habituated. The onset latency of EMG activity in orbicularis oculi was measured in response to 10 stimuli in each subject.

In addition, in three normal subjects the behaviour of the startle response was studied in response to a high (i.e. the stimulus previously defined) and a low intensity auditory stimulus (standardized auditory tone burst of 1000 Hz, 50 ms duration and 60 dB) on different days.

Electrical blink reflexes were obtained in all patients with the SRO syndrome. Twenty trials on each side were recorded in each patient. The stimulus was delivered over the supraorbital nerve every 10 s (duration 0.1 ms at intensities of three to four times threshold, 60–80 V). The latency to onset of unrectified EMG activity for the R1 and R2 components of the blink reflex was measured by visual inspection of single trials on a computer display.

Habituation of the auditory startle response across the three trials in the two groups was examined for each muscle by a repeated measure two-way ANOVA with patient group as the between-subject factor and trial as the within-subject factor. Other statistical analyses were performed using the Mann-Whitney U test for unpaired data and the Wilcoxon signed-ranked test for paired data

RESULTS

Auditory startle response and blink reflex in patients with idiopathic PD and normal subjects

A typical example of normal startle response is shown in Fig. 1A. Group data for normal subjects are summarized in Table 3. These can be compared with the results of the PD group in the 'on' condition, one example of which is shown in Fig. 2A and group results are presented in Table 4. A startle response could be recorded in all normal subjects and patients with PD. However, in both normal subjects and PD patients there was a large variation in the onset latency of the EMG activity in individual muscles during the startle response and considerable variability in the extent of muscle recruitment. When all trials were considered, EMG activity was consistently recorded in only the orbicularis oculi and sternocleidomastoid muscles. Auditory startle responses occurred on more trials in the patients with PD than in the group of normal subjects but this difference was not significant (Wilcoxon paried test $P > 0.05$); in any one muscle, the probability of recording a startle response did not differ between the two groups.

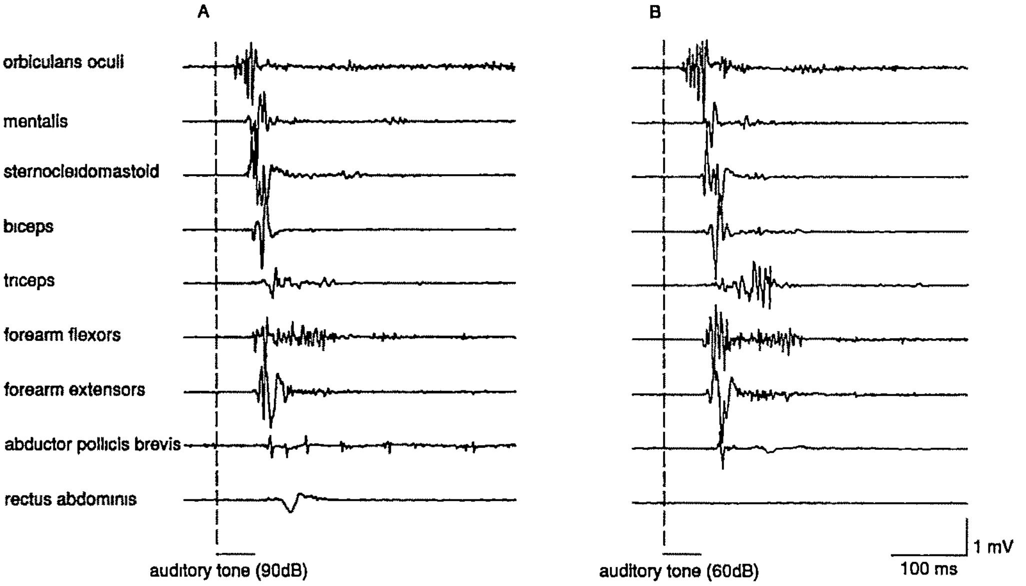

FIG 1. Auditory startle responses in a normal subject following a tone of 1000 Hz for 50 ms at two different intensities of auditory stimulation: 90 dB (A) and 60 dB (B). Single trials are shown of surface EMG recordings from cranial, limb and axial muscles. An early onset EMG burst is present in the orbicularis oculi muscle at about 40 ms which corresponds to the auditory blink reflex. The auditory startle response then follows with a burst of EMG activity in the sternocleidomastoid muscle and a rostral and caudal recruitment (limb and axial) of muscles. The responses to both intensities of auditory stimulation illustrate similar patterns. Note the electrocardiographic signal recorded in the rectus abdominis trace in panel A. Electromyographic responses in this muscle were not present at either intensities of stimulation in this subject (*see* Table 3 for details of the percentage of trials in which responses were recorded in each muscle).

TABLE 3 THE AUDITORY STARTLE RESPONSE IN 12 NORMAL SUBJECTS

Muscle	*Latency (ms)* *Median*	*Range*	*N**	*Percentage***
Orbicularis oculi	40	14–63	12	100
Mentalis	71	47–93	6	55
Sternocleidomastoid	61	42–125	12	98
Biceps	81	72–118	5	26
Triceps	82	67–114	4	26
Forearm flexors	82	41–129	7	40
Forearm extensors	79	41–129	8	43
Abductor pollicis brevis	92	71–136	7	38
Rectus abdominis	94	82–168	8	47

The median latency (ms) and range of latencies to the onset of EMG activity for auditory startle responses in nine different muscles are shown. In some subjects a response was not present on every trial, thus the percentage of trials on which responses occurred may differ from the percentage of subjects who had a response *Number of subjects who had a response on at least one occasion; **refers to total trials on all subjects in which a response occurred.

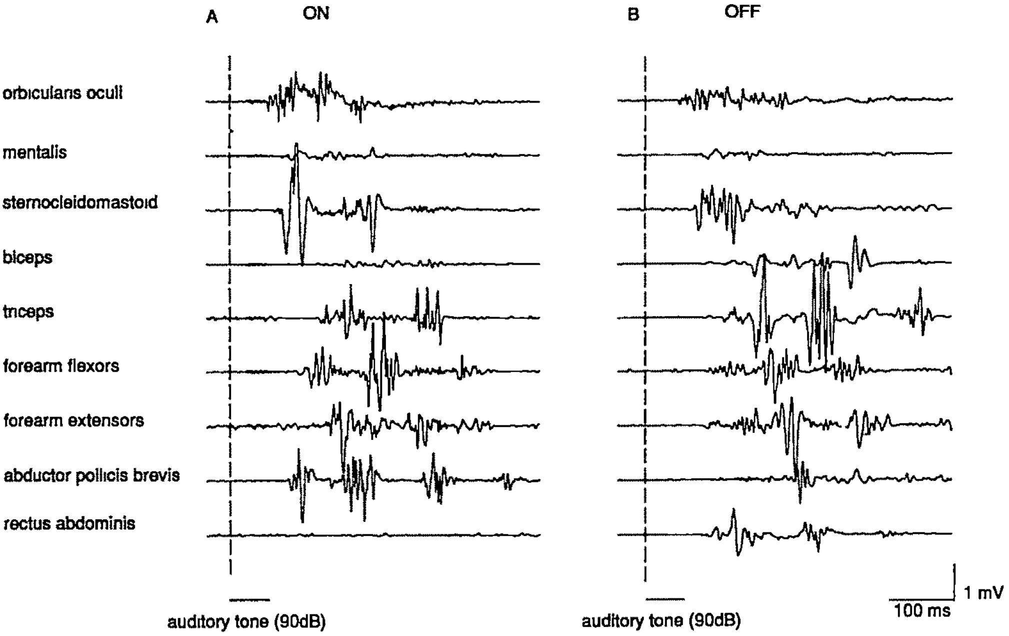

FIG. 2. Auditory startle responses to a tone (1000 Hz, 50 ms duration, intensity 90 dB) in a patient with idiopathic PD (Case 4, Table 1) when 'on' medication and normally mobile (A) and when 'off' medication and akinetic and rigid (B). Single trials are shown. There is no difference in the latency, amplitude or distribution of the responses in either state. However, the onset of EMG activity in orbicularis oculi and sternocleidomastoid is delayed in comparison with normal subjects (*see* text for details).

The sequence of muscle activation in the startle response was similar in patients with PD and normal subjects. The response began with an early burst of EMG activity in the orbicularis oculi muscles, which we have previously suggested may be analogous

TABLE 4 LATENCY TO THE ONSET OF EMG ACTIVITY OF THE AUDITORY STARTLE RESPONSE IN 11 PATIENTS WITH PD IN THE 'ON' STATE

	Latency (ms)			
Muscle	*Median*	*Range*	*N**	*Percentage***
Orbicularis oculi	50	42–64	11	100
Mentalis	87	66–118	7	60
Sternocleidomastoid	77	58–102	11	100
Biceps	85	77–109	7	40
Triceps	94	74–106	6	36
Forearm flexors	95	77–127	8	65
Forearm extensors	102	78–132	8	54
Abductor pollicis brevis	106	53–113	6	37
Rectus abdominis	106	87–143	8	65

*Number of subjects who had a response on at least one occasion, **refers to total trials on all subjects in which a response occurred.

to the auditory blink reflex (Brown *et al.*, 1991*a*). Following this, EMG activity occurred in the sternocleidomastoid and was followed by recruitment of mentalis, arm muscles and rectus abdominis. The difference between the median latencies to onset of EMG activity in sternocleidomastoid and orbicularis oculi (27 ms and 21 ms in patients with PD and normal subjects, respectively), mentalis (10 ms in both groups) and rectus abdominis (28 ms and 33 ms) was similar in the two groups (Mann-Whitney U test $P > 0.05$). There was no habituation in any of the muscles studied across the three trials in either patients with PD or normal subjects (ANOVA $P > 0.05$). The median latency to onset of EMG activity was longer than normal in the orbicularis oculi (Mann-Whitney U test $P < 0.05$) and sternocleidomastoid (Mann-Whitney U test $P < 0.02$) muscles in the patients with PD compared with normal subjects. Responses in the remaining muscles were recorded at comparable latencies in both groups ($P > 0.05$). The delayed onset of EMG activity in orbicularis oculi and sternocleidomastoid muscles in the patients with PD was not due to the small size of the EMG responses since the amplitude of EMG activity in these muscles 50 ms after its onset was comparable in both groups (Mann-Whitney U test $P > 0.05$).

The auditory blink reflex was tested in six of the normal subjects and six patients with PD. The same auditory stimulus was used to evoke the blink reflex and the startle response. The onset latencies of the auditory blink reflex were the same in patients with PD (range 31–49 ms, mean ± SD 38 ± 8 ms) and the normal subjects (range 28–39 ms, mean ± SD 36 ± 4 ms). In normal subjects, the mean latency of orbicularis oculi EMG activity in the auditory blink reflex was also the same as that for the auditory startle response. In the patients with PD, the onset of EMG activity in orbicularis oculi during the startle response was significantly later than that of the auditory blink response (Mann-Whitney U test $P < 0.009$).

Patients with PD 'on' and 'off'

The sequence of activation of different muscles in the startle response was similar before and after treatment with L-dopa (Fig. 2). The characteristics of the EMG response latencies for the different muscles in the five patients 'off' and 'on' are summarized in Table 5. The response latency (Wilcoxon signed-rank test for paired data $P > 0.05$) and amplitude (50 ms after the onset of EMG activity) (Wilcoxon signed-rank test for paired data $P > 0.05$) in all muscles were the same in the 'on' and 'off' states.

TABLE 5. LATENCY TO THE ONSET OF EMG ACTIVITY OF THE AUDITORY STARTLE RESPONSE IN FIVE PATIENTS WITH PD IN THE 'ON' AND 'OFF' STATE

	Latency (ms)					
	Median		*Range*		*N**	
Muscle	*On*	*Off*	*On*	*Off*	*On*	*Off*
Oribularis oculi	59	51	49–64	13–62	5	5
Mentalis	80	65	66–90	65–78	4	3
Sternocleidomastoid	77	72	64–93	45–106	5	5
Biceps	96	87	79–109	82–123	4	3
Triceps	99	86	91–104	68–118	4	2
Forearm flexors	94	79	87–127	64–95	5	4
Forearm extensors	100	76	75–132	64–89	5	4
Abductor pollicis brevis	112	107	53–113	60–125	3	4
Rectus abdominis	106	98	87–128	83–104	4	4

*Number of subjects who had a response on at least one occasion.

Auditory startle responses and blink reflexes in patients with the SRO syndrome

In three patients with the SRO syndrome the auditory startle response was absent. In the remaining five patients in this group, the auditory startle response was present, but abnormal and attenuated. An example is shown in Fig. 3 (Case 1; Table 2) and the group data are summarized in Table 6. The extent of muscle recruitment in SRO syndrome was limited in comparison with normal and varied both from trial to trial and from one patient to the next. In three patients a startle response was obtained in the orbicularis oculi and sternocleidomastoid muscles in every trial. In the remaining two, in whom a startle response was recorded, this only occurred in orbicularis oculi. The median onset latency of EMG activity in orbicularis oculi was similar in patients with the SRO syndrome and PD (around 50 ms). Because of the small number of responses obtained for each muscle, statistical analysis was not performed on the latency and amplitude data, although the latencies of EMG activity in orbicularis oculi, sternocleidomastoid and rectus abdominis muscles in the SRO group appeared to be delayed when compared with the control group (Table 6).

The auditory blink reflex was tested in four patients with the SRO syndrome. In three, the latency was normal and ranged from 31 ms to 49 ms (mean ± SD 40 ± 9 ms). In one patient (Case 8) the auditory blink reflex and startle response were absent.

In order to ensure that the small delayed auditory startle responses in the SRO syndrome were not due to a defect in the perception of the noise, the startle responses were compared with the standard test stimulus and to one of lower intensity in three normal subjects (Fig. 1). There was no significant difference in the latency or amplitude of the startle response to these stimuli.

The blink reflex to electrical stimulation of the supraorbital nerves was examined

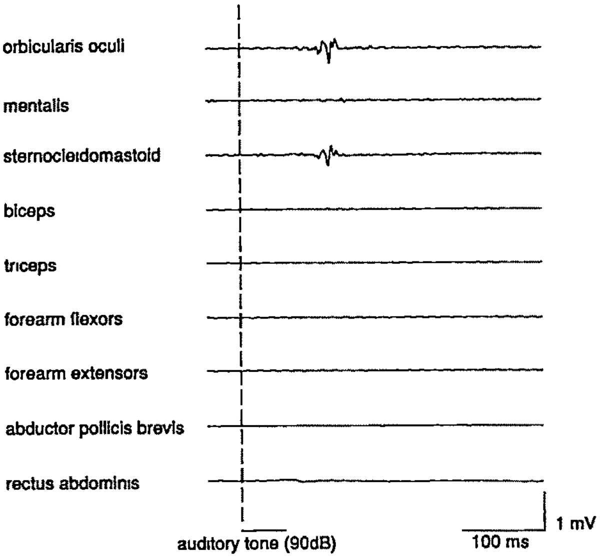

FIG. 3. Auditory startle responses in a patient with a clinical diagnosis of the SRO syndrome (Case 1, Table 2) to an auditory tone (1000 Hz, 50 ms duration, intensity 90 dB) delivered at the time shown. A single trial is shown. The auditory startle response is limited in its distribution with a small delayed response in only the orbicularis oculi and sternocleidomastoid muscles.

TABLE 6 LATENCY OF THE ONSET OF EMG ACTIVITY OF THE AUDITORY STARTLE RESPONSE IN FIVE PATIENTS WITH A CLINICAL DIAGNOSIS OF THE SRO SYNDROME IN THE THREE MUSCLES ACTIVATED

	Latency (ms)			
Muscle	*Median*	*Range*	*N**	*Percentage***
Orbicularis oculi	46	31–108	5	83
Sternocleidomastoid	87	80–131	3	75
Rectus abdominis brevis	139	127–150	2	16

Three other SRO patients had no startle response. The other six muscles shown in Tables 3–5 were silent. *Number of subjects who had a response on at least one occasion, **refers to total trials on all subjects in which a response occurred.

in all eight patients with the SRO syndrome to test the integrity of the brainstem output to the facial muscles. In contrast to the results with the auditory blink reflex, electrically elicited blink reflexes were present and recorded at normal latencies in all eight patients (latency of R1: range 9–13 ms, mean ± SD 11 ± 1 ms; latency R2: range 29–39 ms, mean ± SD 32 ± 4 ms).

DISCUSSION

Steele-Richardson-Olszewski syndrome

A major finding in the present study was that the auditory startle response was often absent in patients with SRO. Even when present, the response was limited in its distribution involving only a few muscles, and usually was delayed in its onset. This abnormality is unlikely to be due to reduced auditory imput. Although there have been reports of perceptive deafness in a few patients with SRO (Lees, 1987), auditory evoked potentials usually are normal (Tolosa and Zeese, 1979), and in our patients hearing was clinically normal. Since the auditory startle response in normal subjects was not affected by reducing the intensity of the auditory stimulus it is unlikely that slight deafness would have a great effect on the startle response. We conclude that the disturbance of the auditory startle response in patients with SRO was not due to an abnormality of the primary auditory input. Similarly, there was no evidence of an abnormality of the bulbar lower motor neuron pathways since the electrical blink reflex was normal. These results suggest that loss of the auditory startle response in patients with the SRO syndrome is caused by a disease of the central structures involved in the response. This conclusion is consistent with the known pathology of the SRO syndrome. Quantitative immunocytohistochemical evaluation of brainstem cholinergic cells in SRO using polyclonal antibodies against human choline acetyltransferase reveals loss of cholinergic neurons in the lower pontine reticular formation in SRO, mainly within the cells of the nucleus pontis centralis caudalis and the nucleus papillioformis (Juncos *et al.*, 1991; Malessa *et al.*, 1991). These nuclei occupy a portion of the human brainstem which is coextensive with corresponding nuclei defined in animals such as the nucleus reticularis pontis centralis caudalis and the nucleus reticularis tegmenti (Olszewski and Baxter, 1954; Taber, 1961). The nucleus reticularis pontis centralis caudalis is directly involved in the startle reflex of the rat (Davis 1982), and we suggest that the absent or poor auditory startle responses in patients with SRO could be explained by the pathological lesion of this part of the pontine reticular formation.

The auditory blink reflex was also absent in those patients without an auditory startle reflex. The relationship between the auditory blink reflex and the auditory startle response is unclear. Some authors consider the auditory blink to be part of the startle response (Landis and Hunt, 1936, 1937; Szabó and Hazafi, 1965; Gogan, 1970; Wilkins *et al.*, 1986), but Brown *et al.* (1991*a*) have provided evidence suggesting that the auditory blink reflex involves mechanisms separate from those responsible for the auditory startle response. However, the present results suggest that the two responses use pathways which occupy similar anatomical locations within the brainstem.

In contrast to the results with the auditory startle response and the auditory blink reflex, electrically elicited blink reflexes were present and of normal latency in all patients with the SRO syndrome. If the abnormalities of the auditory startle response and auditory blink reflex are due to lesions of central brainstem pathways, then the present results suggest that the central pathways mediating the electrical blink reflex are not the same as those of the auditory blink reflex.

Parkinson's disease

In patients with PD, the form of the auditory startle response, and the muscles recruited were the same as in normal subjects. This indicates that the neuronal circuits mediating the auditory startle response are intact in PD. However, the present results suggest that changes in basal ganglia function may influence the timing of EMG responses recruited in the auditory startle as the onset of the auditory startle response was delayed in orbicularis oculi and sternocleidomastoid in comparison with the control group. One explanation for this might be a lack of facilitation onto the startle system within the reticular formation, perhaps due to abnormal basal ganglia output in PD (Alexander and Crutcher, 1990).

The question arises as to whether changes in this aspect of basal ganglia output in PD can be linked to the disorders of the startle response seen in the present experiments. It has been proposed that the basal ganglia output to brainstem nuclei is involved in the control of normal posture and locomotion (Coles *et al.*, 1989; Garcia-Rill, 1991) and that disturbances of this projection give rise to the postural instability seen in PD (Hirsch *et al.*, 1987). The auditory startle response is known to be modified by changes in posture; leg muscle EMG activity in the auditory startle response occurs some 40–60 ms earlier when the subject is standing than when sitting relaxed (Brown *et al.*, 1991*c*). This observation prompted the suggestion that the auditory startle response might be conducted as a series of waves of bulbospinal activity. Changes in the latency of leg muscle responses with alterations in body posture could then be explained by facilitation of an earlier wave by postural activity. Evidence for abnormal descending reticulospinal function in PD was advanced by Delwaide *et al.* (1990, 1991), who demonstrated a reduction in the amount of audiospinal facilitation of the soleus H-reflex in patients with PD (using a similar auditory stimulus). In addition, the timing of maximal facilitation of the soleus H-reflex in patients with PD was delayed in comparison with control subjects.

Little clinical information is available about the descending projections from the globus pallidus and the substantia nigra pars reticulata to brainstem nuclei. Clinical observations suggest that the functions of these pathways are not influenced by L-dopa. For example, at least in the elderly or in advanced disease, L-dopa has little effect on postural control in patients with PD, despite its dramatic influence on other aspects of movement (Lakke, 1985; Weiner and Lang, 1989). In keeping with this was the absence of any detectable effect of L-dopa treatment on the latency of the startle response in any of the five patients with PD who were studied 'on' and 'off' despite a clear improvement in their motor performance. Experiments in normal animals also have demonstrated little or no influence of L-dopa (Astrachan and Davis, 1981; Kokkinidis and MacNeill, 1982) or apomorphine (Astrachan and Davis, 1981; Svensson, 1990) on the auditory startle response. Other possible explanations for the delayed startle responses in patients with PD include the influence of cerebral cortical projections onto the startle reflex pathway, although these are not essential for startle. Startle responses have been obtained in chloralose anaesthetized or in decorticated or decerebrated animals (*see* Davis *et al.*, 1982 for a review). Another factor is the level of arousal of the patients. It may have been that the patients were less alert than normal. However, arousal mainly affects the size of the response, with the startle being smaller in less alert subjects (Huttenlocher, 1960). Since the size of the startle response was normal in our patients, arousal is unlikely to have been an important influence on the results.

In contrast to the startle response, the auditory blink reflex in orbicularis oculi was of normal latency in patients with PD. This was an odd finding since both types of response were elicited by the same auditory stimulus, the only difference between them being that the stimulus was given much more frequently in the auditory blink paradigm than during the startle response. Why repetition rate should influence the timing of orbicularis oculi contraction in PD is unclear. It may be that when auditory stimuli are given frequently there is a relative facilitation of the orbicularis oculi reflex pathway which is lacking when stimuli are given every 20 min.

In conclusion, in patients with SRO the startle response is abnormal or absent. We suggest that this may be related to the pathological lesion of the pontine reticular formation, mainly in the nucleus reticularis pontis caudalis which is thought to be an important relay in the startle response. In contrast, the startle response in patients with PD is of normal size but delayed, findings that may be related to withdrawal of facilitatory input to brainstem centres from the basal ganglia.

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CHARACTERIZATION OF CONTRALATERAL TORQUES DURING STATIC HIP EFFORTS IN HEALTHY SUBJECTS AND SUBJECTS WITH HEMIPARESIS

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SUMMARY

Contralateral torques exerted at the hip were measured in healthy subjects and subjects with hemiparesis performing unilateral static hip efforts in abduction, adduction, flexion and extension, in a sitting position, at two torque levels. In general, the ipsilateral hip efforts were accompanied by mirrored contralateral torques in both groups of subjects. The directionality of these contralateral torques indicates that their action at the pelvis is mechanically opposite to the ipsilateral efforts, suggesting that they ensure the stabilization of the pelvis. In healthy subjects, analyses of variance showed no difference in the magnitude of the contralateral torques with regard to which limb was used to perform the task. However, a significant increase in magnitude was demonstrated in the contralateral torques concurrent with the increasing level of effort requested ipsilaterally. In hemiparetic subjects, when performing the tasks with their paretic limb, the magnitude of the contralateral torques was significantly increased in the non-paretic limb when compared with those measured in the paretic limb during non-paretic limb efforts. Based on the present results, a model of postural control is presented to explain the relationship between the ipsilateral and contralateral torques. Using this model, it is hypothesized that the increased contralateral torques observed in hemiparetic subjects when performing the tasks with their paretic limb is related to the weakness of the paretic muscles. The clinical importance of exercises used for the re-education of the paretic lower limb in this population, which consist of resisting the non-paretic hip movements in order to strengthen the paretic hip muscles, is discussed in light of these results.

INTRODUCTION

Raïmiste (1909, 1911) studied the contralateral muscular activity occurring at the hip joint during unilateral static hip efforts in a group of healthy subjects and a group of subjects with hemiparesis. He reported that with healthy subjects in the supine position, static hip abduction and adduction efforts generated activity in the contralateral hip abductor and adductor muscles, respectively, and that static hip flexion and extension efforts were associated with activity of the contralateral hip extensor and hip flexor muscles, respectively. He proposed that these contralateral forces were generated in order to stabilize the pelvis during an ipsilateral hip effort. Indeed, a hip torque of the same magnitude produced contralaterally, in a direction opposite to the ipsilateral effort, would neutralize the displacements of the pelvis caused by the ipsilateral effort. In healthy

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subjects, these contralateral muscular contractions did not result in any visible movements of the limb. In fact, if the contralateral muscular activities produced any perceptible movement, Raïmiste (1909) reported that the subject could voluntarily control it by contracting the muscles that are antagonist to the ones implicated in the described patterns. In a subsequent paper, he suggested that the hip and back extensor muscles were also used to stabilize the contralateral limb during hip abduction and adduction efforts. The activation of these muscles permitted the pressing of the lower extremity into the bed to provide further resistance to movement due to the force of friction (Raïmiste, 1911).

For hemiparetic subjects, Raïmiste (1909, 1911) observed patterns of contralateral muscular activity that were similar to those described in healthy subjects. However, he noted that the contralateral muscular activations during non-paretic hip exertions caused movements of the paretic leg which could not be stopped voluntarily. This is in opposition to what was seen when resisting the paretic limb or for healthy subjects. Raïmiste (1909, 1911) concluded that for hemiparetic subjects, the muscles responsible for limb stabilization were too weak on the paretic side to prevent movement from occurring. These observations reported by Raïmiste for hemiparetic subjects are of importance since they have been incorporated into a therapeutic evaluation and treatment programme developed for the hemiparetic population to strengthen the paretic muscles (Brunnstrom, 1970). Part of this treatment programme consists of applying resistance to the non-paretic hip abduction or adduction movements to facilitate the activation of the contralateral paretic hip abductor or adductor muscles, respectively.

The aim of this study was to quantify the contralateral torques generated during unilateral hip efforts in a group of healthy subjects and a group of subjects with hemiparesis, using a controlled experimental situation involving a distal fixation of both thighs. It was hypothesized that appropriate patterns of contralateral torques would be generated to provide stabilization of the pelvis in both healthy and hemiparetic subjects, and furthermore, that the magnitude of the contralateral torques would be different in hemiparetic subjects depending on whether the effort is made either with the paretic or with the non-paretic limb.

MATERIAL AND METHODS

Population characteristics

The first group of subjects consisted of 18 individuals (12 women, six men) with no history of neurological problems or any known lower extremity injury or disease. The mean age for this control group was 37 yrs (range 23–59 yrs, SD = 13). The second group of subjects consisted of 16 individuals (seven women, nine men) who had sustained a cerebrovascular accident (CVA). Their mean age was 49 yrs (range 21–79 yrs, SD = 18) with the mean post-stroke delay of 18.6 mths (range 2–45 mths, SD = 18.1). Subjects with hemiparesis were recruited from two rehabilitation centres and were selected according to the following criteria: (i) paresis of the lower extremity resulting from a first unilateral CVA; (ii) good endurance in the sitting position; (iii) no severe perceptual, cognitive or comprehension deficits; (iv) absence of severe sensory deficits; (v) absence of significant contracture to the lower extremities; (vi) continence. Before the testing procedure, the global motor function was evaluated for each subject with hemiparesis, using an evaluation of physical performance (Fugl-Meyer *et al.*, 1975). Muscle tone of the paretic lower extremity was evaluated on a six-point scale (0, 1, 1+, 2, 3, 4 from 0 = normal tone to 4 = severe spasticity) which is the Ashworth scale (Ashworth, 1964) modified by Bohannon and Smith (1987). Both clinical tests have previously been documented for their validity and reliability (Duncan *et al.*, 1983, Bohannon and Smith, 1987). A summary of the clinical status of the subjects with hemiparesis appears in Table 1. Each subject gave informed consent prior to the experiment.

TABLE 1 DESCRIPTIVE CHARACTERISTICS OF SUBJECTS WITH HEMIPARESIS

Subjects	*Age (yrs)*	*Delay post-stroke (mths)*	*Sex*	*Paretic side*	*Fugl-Meyer (total) /242*	*Ashworth (lower extremity) 0–4*
1	44	4	M	R	174	1+
2	22	44	F	L	209	1+
3	34	39	M	R	156	0
4	52	4	M	L	136	0
5	42	4	M	L	121	2
6	24	5	F	L	192	2
7	24	4	F	R	131	1
8	67	36	M	L	224	1
9	32	6	M	R	180	0
10	69	5	M	L	178	1
11	68	42	F	R	138	2
12	47	45	F	L	156	1
13	50	45	F	R	189	1
14	68	3	M	L	116	0
15	62	9	M	L	195	0
16	79	3	F	R	163	0

R = right; L = left

Experimental arrangement and data processing

Two static multidirectional dynamometers for the lower extremity were used to measure simultaneously torques exerted at the hips and knees (Fig. 1). Subjects were seated in a chair with the trunk secured by straps, and were positioned with their hips at approximately 80° and their knees at 90° (anatomical position corresponding to 0°). The lower limbs of the subjects were enclosed within fixation rings at the distal thigh and at the leg. Sensors measured orthogonal forces exerted at the fixation rings. These forces were digitized at 50 Hz using an analogue to digital conversion card (Tecmar, model PGH) and recorded on a desktop computer (IBM, AT). Lever arms identified in Fig. 1 were measured at the beginning of each experiment and were entered into a software program. The forces and lever arm values were used to calculate muscular torques exerted in the different anatomical planes at the hips and knees according to the equations of static equilibrium indicated in Fig. 1. Torques were calculated in flexion/extension, abduction/adduction and internal/external rotation of the hips and in flexion/extension of the knees. The validity and reliability characteristics of the apparatus have been documented previously (Gauthier *et al.*, 1990). The torques generated by the weight of the limbs were controlled for at the beginning of each experimental session.

Procedure and experimental design

During the experimental procedures, subjects were provided with visual feedback concerning the magnitude and direction of hip exertions. They were seated in front of a monitor on which a mobile cursor and a target were displayed. A hip flexion effort displaced the arrow upwards while a hip extension effort displaced it downwards. The arrow moved sideways during hip abduction efforts (to the left for the left lower limb and to the right for the right lower limb), and during hip adduction efforts (to the right for the left lower limb and to the left for the right lower limb). The displacement of the arrow was proportional to the hip torque exertion. Subjects were asked to maintain a given torque level in the appropriate direction by placing the arrow within the target and maintaining it for a period of 1 s. The targets represented either a hip flexion, extension, abduction or adduction effort and were presented in a set order. Each task was performed twice by healthy subjects and by subjects with hemiparesis when using their non-paretic limb. In order to prevent fatigue, each task was accomplished only once by the subjects with hemiparesis when using their paretic limb. While achieving the tasks, feedback was given exclusively in the hip plane in which the task was performed (e.g. hip flexion-extension plane). However, torques exerted in all anatomical planes from both the hips and knees were recorded when the task was successful.

The magnitude of the two torque levels requested (levels 1, 2) for women and men in all directions

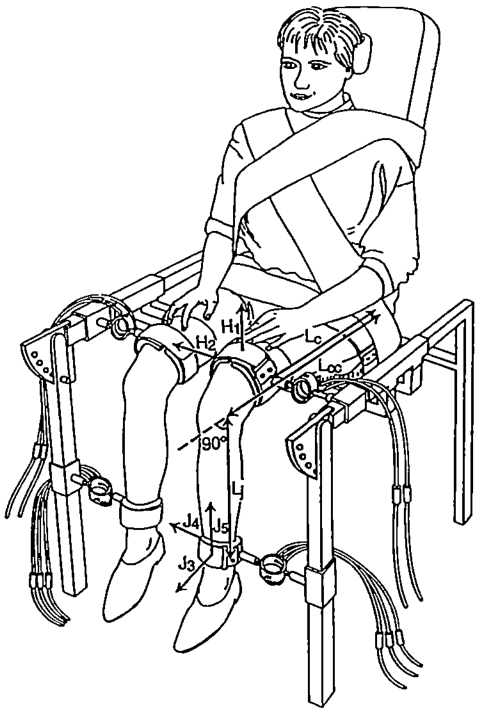

FIG. 1 General overview of the two static multidirectional dynamometers used to measure the torques exerted simultaneously at both hips and knees. Appropriate lever arms (Lc, Lcc and Lj) are measured for each subject. The torques in hip flexion/extension, abduction/adduction and internal/external rotation and in flexion/extension of the knee are calculated using the static equilibrium equations

$$\text{Torque flexion/extension (hip)} = H_1\ Lc + J_3\ Lcc \cos\phi + J_3\ Lj + J_5\ Lcc \sin\phi$$

$$\text{Torque abduction/adduction (hip)} = H_2\ Lc + J_4\ Lcc + J_4\ Lj \cos\phi$$

$$\text{Torque internal/external rotation (hip)} = J_4\ Lj \sin\phi$$

$$\text{Torque flexion/extension (knee)} = J_3\ Lj$$

are detailed in Table 2. These values represented 5% and 15% of the mean maximal voluntary contraction measured in the different hip anatomical planes for four healthy women in a preliminary study. For the men of both samples, the magnitude of these torques were systematically increased by 30% with respect to the required torques for the women. These percentages (5% and 15%) were determined by trial and error and were chosen with the understanding that all subjects with hemiparesis would be able to achieve a majority of tasks at torque level 1 when using their paretic limb. Therefore, at torque level 2, tasks were performed with both limbs in healthy subjects and with the non-paretic limb only for hemiparetic subjects. However, it should be noted that the group of hemiparetic subjects studied could, in fact, generate the higher torque level but experienced difficulty controlling and maintaining the direction and magnitude of the efforts requested. Subjects with hemiparesis performed the tasks using their non-paretic limb first while healthy subjects began the session with their right lower extremity. To prevent muscle fatigue, sufficient rest periods were given between trials when necessary.

Statistical analysis

The resulting torque values obtained in healthy and hemiparetic subjects were first analysed statistically using a Student *t* test to determine if the torques were significantly different from zero. Two-way analyses

TABLE 2 MAGNITUDE OF TORQUES (NANOMETRES) REQUESTED DURING THE TASKS IN HIP FLEXION, EXTENSION, ABDUCTION AND ADDUCTION FOR WOMEN AND MEN AT TORQUE LEVELS 1 AND 2

		Hip flexion	*Hip extension*	*Hip abduction*	*Hip adduction*
Women	Level 1	4 63	5.01	3 44	2.36
	Level 2	13.89	15 03	10.33	7.17
Men	Level 1	6.02	6.51	4.47	3.07
	Level 2	18.06	19.54	13.43	9.32

of variance (ANOVA) with repeated measures were used for healthy subject data to test for the effect of the limb used to perform the tasks (left vs right) and the effect of the level of effort performed ipsilaterally (level 1 vs 2) on the contralateral associated torques. For repeated measures on one-factor ANOVA were also used to test the effect of lower limb used to perform the tasks on the contralateral torques in healthy and hemiparetic subjects at torque level 1. In addition, the direction and magnitude of contralateral level 2 torques associated with non-paretic limb efforts were compared with those associated with right and with left limb performances, respectively, in healthy subjects using Student *t* tests. A level of probability of 0.05 was considered as significant. Only the contralateral torques present in the patterns described by Raïmiste (1909, 1911) are presented and analysed in this paper. In these analyses, the hip torques in flexion and in adduction were arbitrarily given positive values while torques in the opposite directions were given negative values.

RESULTS

Patterns of contralateral torques during static hip efforts in healthy subjects

In healthy subjects, specific torque patterns were observed at the contralateral hip during unilateral static hip efforts regardless of which lower limb performed the tasks. During a hip abduction effort, a contralateral hip abduction torque significantly different from zero ($P < 0.01$) was generated at both torque levels (Fig. 2).

Similarly, a hip adduction effort was associated with a contralateral hip adduction torque (Fig. 3). While efforts in these directions involved the activation of the bilateral homologous muscles, hip efforts performed in flexion or extension contralaterally recruited the antagonist muscles. Indeed, hip flexion and hip extension efforts were associated with contralateral hip extension and hip flexion torques, respectively, at both torque levels (Fig. 3).

Magnitudes of the contralateral torques in healthy subjects

The results of the statistical analyses indicated no significant differences ($P > 0.05$) between the magnitude of the contralateral torques produced during right and left limb exertions in healthy subjects in all but one condition (Table 3). The exception was observed during the hip abduction efforts, where the magnitude of the contralateral hip abduction torques associated with right limb exertions was greater than those associated with left limb efforts for both torque levels (Fig. 2). Furthermore, these analyses showed that the magnitude of all the contralateral torques was significantly increased ($P < 0.05$) during efforts made at torque level 2 as compared with torque level 1 (Table 3). The only exception being the contralateral hip flexion torque associated with a hip extension exertion, which was not significant ($P > 0.05$).

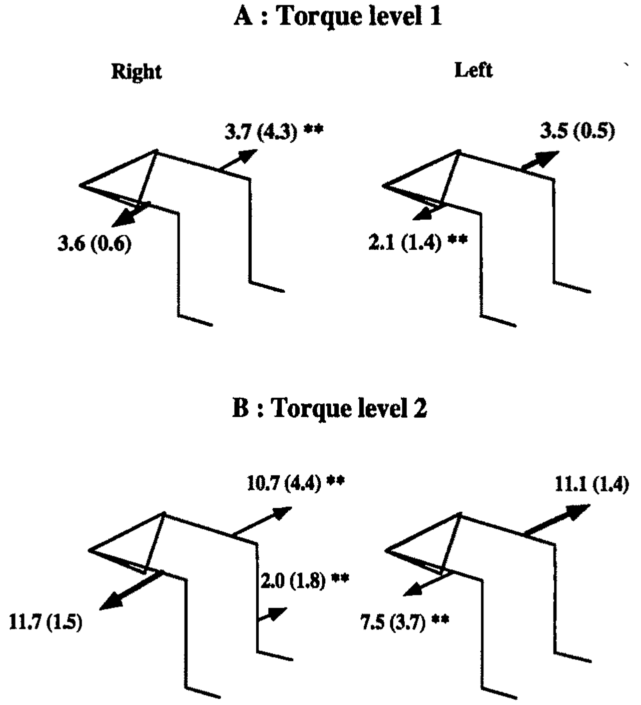

FIG. 2. Torques (mean ± 1 SD) (Nm) associated with a hip abduction effort performed by healthy subjects (n = 18) using the right and the left lower extremities at torque level 1 (A) and torque level 2 (B) Thick arrows represent the tasks accomplished and the fine arrows reflect the associated torques. $^{**}P < 0.01$

Patterns of contralateral torques during static hip efforts in hemiparetic subjects

Patterns of contralateral torques similar to those already described for healthy subjects were observed for subjects with hemiparesis. For example, contralateral abduction torques significantly different from zero ($P < 0.05$) were measured during paretic and non-paretic hip abduction efforts at torque level 1 (Fig. 4).

Patterns similar to those described for healthy subjects were also present during both hip exertions for all directions tested except for the paretic hip extension task (Fig. 5). In fact, a hip extension effort performed with the paretic limb was associated contralaterally with a hip extension torque as opposed to a hip flexion torque during non-paretic limb exertion and for healthy subjects.

Magnitudes of the contralateral torques in hemiparetic subjects

The magnitude of the contralateral torques measured during paretic limb performance was significantly increased ($P < 0.05$) for all tasks compared with those observed during non-paretic limb exertions (Table 4). The only exception was the contralateral hip extension torque associated to a hip flexion effort. In this condition, the difference between paretic and non-paretic limb exertions was not significant ($P > 0.05$).

Hemiparetic subjects also accomplished the tasks at torque level 2 with their non-paretic

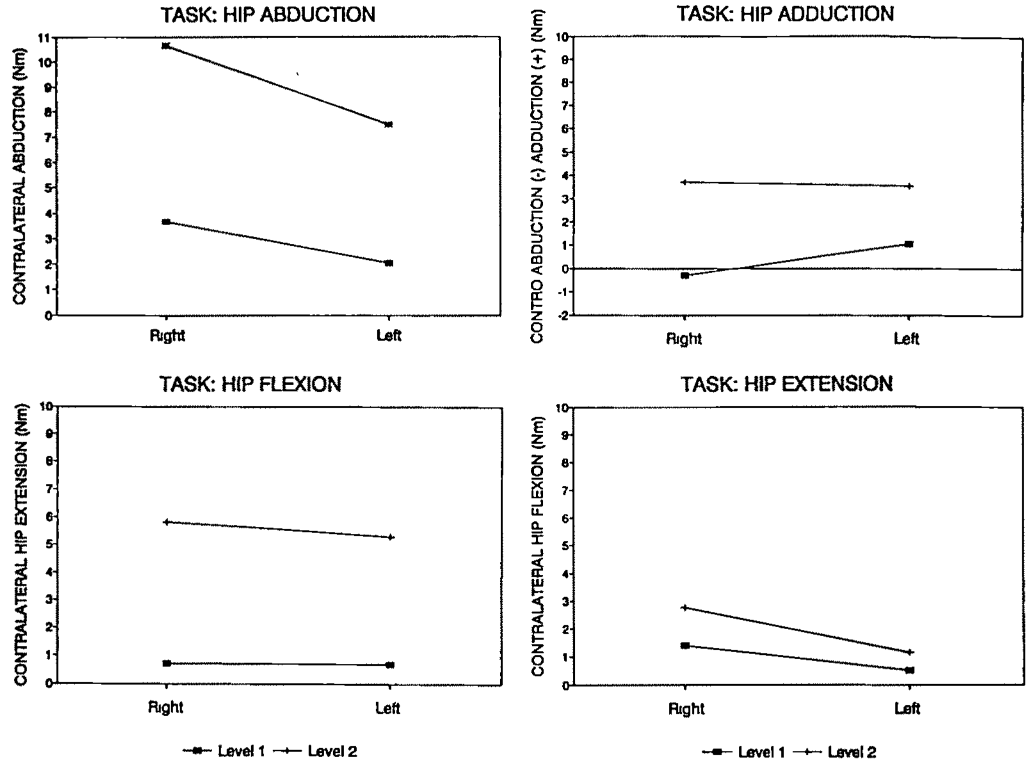

FIG. 3. Mean contralateral torques (Nm) associated with hip abduction, adduction, flexion and extension efforts performed using the right and the left lower extremities at torque level 1 and torque level 2 in healthy subjects (n = 18). The labels clarify the side on which the task was performed

TABLE 3. SUMMARY OF TORQUE LEVEL AND LIMB EFFECTS IN HEALTHY SUBJECTS

Task	*Contralateral torque*	*Effect of level*		*Effect of limb*		*Interaction*	
		F	*P*	*F*	*P*	*F*	*P*
Hip flexion	Hip extension	29.89	0.0001	0.08	0.7796	0.11	0.7486
Hip extension	Hip flexion	3.10	0.0962	2.04	0.1711	0.72	0.4079
Hip abduction	Hip abduction	222.51	0.0001	4.86	0.0416	4.58	0.0471
Hip adduction	Hip adduction	45.58	0.0001	0.28	0.6022	10.38	0.0050

Results of ANOVA for repeated measures testing the effect of torque level performed and of the lower limb used to perform the task on associated contralateral torques in healthy subjects (n = 18)

limb (Fig. 6). Results showed that the magnitude of the contralateral torques measured at the paretic hip had a tendency to be smaller than those observed in healthy subjects during right or left limb performances. However, statistical analyses (Table 5) showed that in most conditions (six out of eight) the contralateral torques were not significantly different ($P > 0.05$) between healthy and hemiparetic subjects. These results suggested that contralateral torques associated with non-paretic limb efforts in hemiparetic subjects were not different from those seen during healthy subjects' exertions.

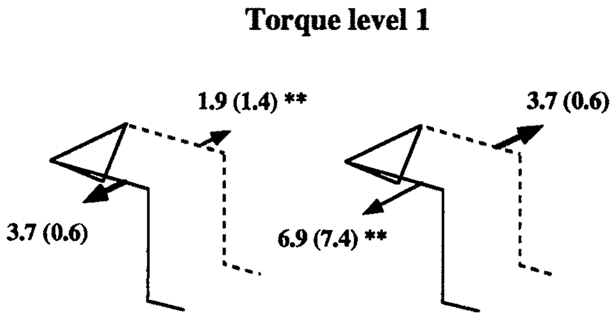

FIG. 4. Torques (mean ± SD) (Nm) associated with a hip abduction effort performed by hemiparetic subjects (n = 16) using their paretic and non-paretic lower limbs at torque level 1. The dotted lines represent the paretic limb Thick arrows represent the tasks accomplished and the fine arrows reflect the associated torques $^{**}P < 0.01$

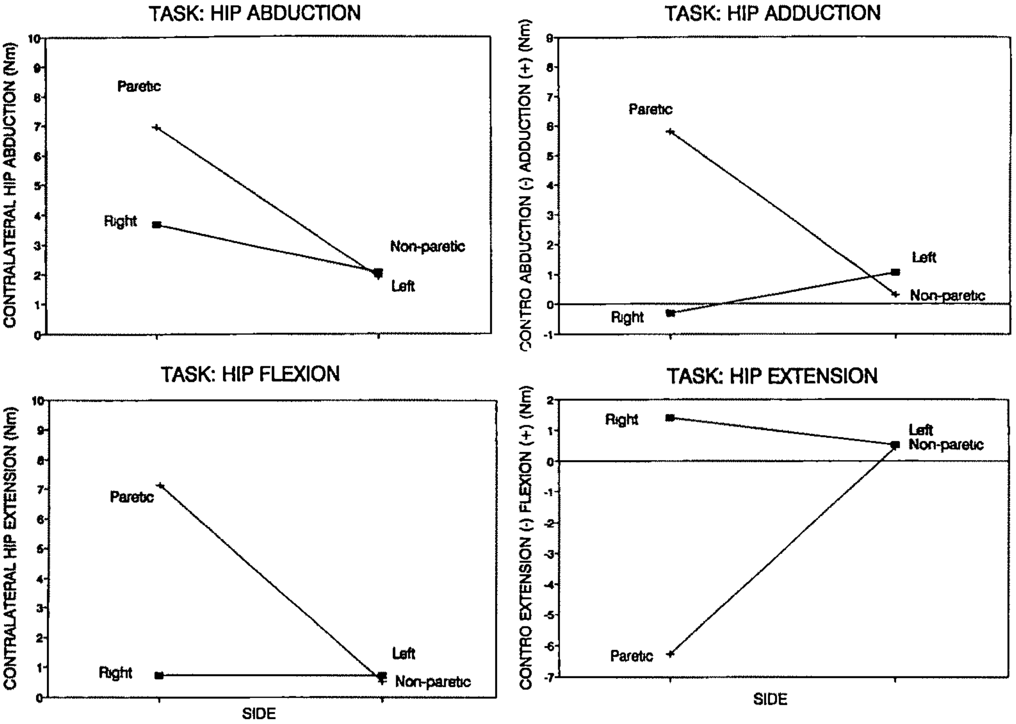

FIG. 5. Mean contralateral torques (Nm) associated with hip abduction, adduction, flexion and extension efforts in healthy subjects (n = 18) using their right and left lower limbs (■) and in hemiparetic subjects (n = 16) using their paretic and non-paretic lower extremities (+) at torque level 1. The labels clarify the side on which the task was performed.

DISCUSSION

Contralateral hip torques were measured in healthy and hemiparetic subjects during unilateral static hip exertions. The results supported the hypothesis suggesting that contralateral hip torque patterns are generated during unilateral hip exertions in order

TABLE 4. SUMMARY OF LIMB EFFECTS IN HEALTHY AND HEMIPARETIC SUBJECTS

Task	Controlateral torque	Effect of limb (normals)		Effect of limb (hemiparetics)	
		F	*P*	*F*	*P*
Hip flexion	Hip extension	0.01	0.9312	4.29	0.0560
Hip extension	Hip flex./ext.	2.50	0.1325	7.56	0.0166
Hip abduction	Hip abduction	2.31	0.1466	6.04	0.0288
Hip adduction	Hip adduction	1.79	0.1983	17.36	0.0008

Results of ANOVA for repeated measures on one-factor testing the effect of the lower limb used to perform the task in healthy subjects (n = 18) and in hemiparetic subjects (n = 16) at torque level 1.

to provide stabilization of the pelvis. Moreover, the results showed that the magnitude of the contralateral torques is different in hemiparetic subjects depending on the limb used to perform the tasks; the contralateral torques being significantly increased during paretic hip exertions compared with those measured during non-paretic hip exertions and for healthy subjects.

Purpose of the contralateral torques

Stabilization of the pelvis using abdominal muscles (Walters and Partridge, 1957; Sheffield, 1962; Flint, 1965; Flint and Gudgell, 1965; Lipetz and Gutin, 1970; Carman *et al.*, 1972; de Sousa and Furlani, 1974; Bankoff and Furlani, 1984), dorsal muscles (Fischer and Houtz, 1968) or contralateral leg muscles (Raïmiste, 1909, 1911) have been observed during hip movements or hip efforts in healthy subjects. Our results support the hypothesis that associated contralateral torques are used to provide postural stability of the pelvis and ensure a mechanical support for hip movements or efforts (Raïmiste, 1909, 1911). For example, a right hip abduction exertion causes a counter-clockwise rotational force at the pelvis. A synchronous contraction of the contralateral hip abductors of the same magnitude provides stability by neutralizing the forces at the pelvis. A similar principle would apply to other hip efforts. The directionality of the contralateral torques measured was such that appropriate postural stabilization of the pelvis was achieved. Strategies used for pelvis stabilization included the bilateral activation of homonymous muscles as during hip abduction or adduction efforts or the bilateral activation of antagonist muscles as during efforts in hip flexion or extension. It is suggested that these contralateral torques are generated to provide the postural adjustments required during a unilateral hip effort. The magnitude of the contralateral torques was generally smaller than the ipsilateral torques produced in healthy subjects and in hemiparetic subjects during non-paretic limb exertions. This may be explained by the friction and reactive forces caused by the chair which provide some inherent stability of the pelvis.

One exception to the hypothesis that the contralateral torques played a mechanical role in the stabilization of the pelvis was observed. In hemiparetic subjects, a hip extension effort of the paretic limb was associated to a contralateral hip extension torque, instead of a contralateral hip flexion torque as seen during non-paretic limb of healthy subject performances. However, it is possible that this change of strategy in hemiparetic subjects resulted from the weakness of the hamstring muscles on the paretic side. Indeed, it is

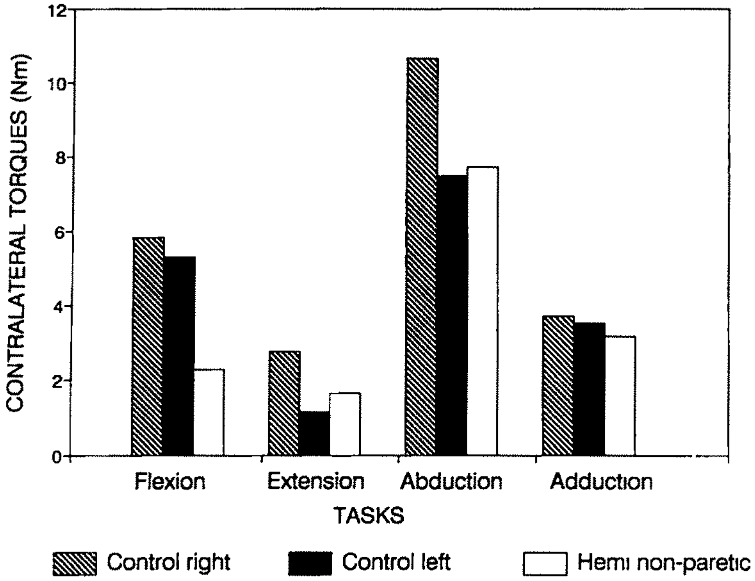

FIG. 6. Mean contralateral torques (Nm) associated with hip flexion (contralateral hip extension), extension (contralateral hip flexion), abduction (contralateral hip abduction) and adduction (contralateral hip adduction) efforts in healthy subjects (n = 18) and hemiparetic subjects (n = 16). The labels clarify the side on which the task was performed (right, left sides for healthy subjects and non-paretic side for hemiparetic subjects) at torque level 2.

TABLE 5 SUMMARY OF GROUP EFFECT

Task	*Contralateral*	*Normal (right)* *P*	*Normal (left)* *P*
Hip flexion	Hip extension	0.08	0 02
Hip extension	Hip flexion	0 46	0.85
Hip abduction	Hip abduction	0.05	0.88
Hip adduction	Hip adduction	0.73	0.31

Results of Student *t* tests comparing the contralateral torques measured in subjects with hemiparesis (n = 16) when performing the tasks with their non-paretic limb to contralateral torques in healthy subjects (n = 18) using their right limb and using their left limb to perform the tasks at torque level 2

possible that producing a symmetrical effort facilitates the activity of the contralateral paretic muscles. Alternatively, the bilateral exertion may result in lifting the pelvis and therefore allowing the subject to support some body weight over their thigh rather than applying a muscular force. Both assumptions suggest that the change of strategy probably resulted from weakness of the paretic muscles.

Implication of the results for the Raïmiste phenomena

Raïmiste (1911) and Noïca (1911) suggested that the contralateral limb movement observed in hemiparetic subjects during non-paretic hip efforts was caused by the weakness of the paretic muscles that are antagonist to the ones implicated in the patterns.

Furthermore, other authors (Cacciapuoti, 1911; Marie and Foix, 1916) believed that, in addition to the weakness of these muscles, the activity of the paretic muscles participating in the patterns would be increased, as compared with healthy subjects, and would contribute to the movements observed on the paretic side. Whether only one or both of these mechanisms are responsible for the contralateral movements seen in hemiparetic subjects during non-paretic hip efforts has never been clarified. Our results showed that the torques at the paretic limb during non-paretic limb exertions are not increased compared with those measured in healthy subjects. In light of these results, it is postulated that the main cause of the movements observed at the paretic limb by Raïmiste during non-paretic limb efforts is the weakness of the paretic muscles that are antagonist to the ones implicated in the patterns as originally hypothesized by Raïmiste (1909, 1911) rather than the increased activity of the contralateral paretic muscles implicated in the patterns.

The results of this study also demonstrated increased torques at the non-paretic limb during paretic limb exertions. It appears that these increased torques could not be observed by Raïmiste since hemiparetic subjects provided postural stabilization by using the non-paretic hip and back extensor muscles to press the lower limb into the bed as well as the muscles antagonist to the ones implicated in the patterns. Using this strategy, Raïmiste (1911) reports that hemiparetic subjects could voluntarily fix their contralateral non-paretic limb during efforts on the paretic side.

Central organization of the coordination between ipsilateral and contralateral torques in healthy subjects

If one accepts the premise that contralateral torques were generated to stabilize the pelvis, bilateral coordination could be regulated by way of a postural control mechanism. Different postural control mechanisms could explain the coordination between these bilateral torques. It is possible that two independent commands are sent to the ipsilateral and contralateral muscles to generate the bilateral torques. However, the observation that the amplitude of the contralateral torques varies according to the torque level exerted ipsilaterally suggests that the commands sent to the ipsilateral and contralateral muscles are closely related. In addition, preliminary results obtained in our laboratory, in both healthy and hemiparetic subjects, indicate that bilateral torques co-vary closely in time.

A different postural control model consisting of a common command sent bilaterally could better explain the coordination between the ipsilateral and contralateral torques. In this model, a common command sent to homonymous muscles in the case of the hip abduction and adduction tasks or to antagonist muscles for the hip flexion and extension tasks would result in similar torques bilaterally. Although this model could explain the results obtained in healthy subjects, it is limited in its ability to explain the results obtained in hemiparetic subjects. Based on the evidence that hemiparetic subjects demonstrate motor weakness of their paretic muscles (Tang and Rymer, 1981; Adams *et al.*, 1990), a common command scaled to produce a given torque on the paretic side would result in an increased torque on the non-paretic side, as it was observed in the results. However, when this common command is scaled to produce a given torque on the non-paretic side, because of the weakness of the paretic muscles, a lower value of torque should be measured on that side. Although this tendency was observed, the statistical analyses demonstrated that the torques at the paretic limb during non-paretic limb efforts were not significantly different compared with those measured in healthy subjects. This observation suggests that a more complex mechanism model exists.

A model described by Paulignan *et al.* (1989) which includes two parallel commands, one controlling the ipsilateral effort and the other controlling the contralateral postural adjustment, that are linked together by an internal collateral circuit including a gain control (Fig. 7A), will be used to explain the results of this study. When healthy subjects perform a specific task with the left limb (Fig. 7A), a central motor command gives rise to an ipsilateral torque. It contributes also, via the internal collaterals, to the gain control for the generation of an appropriate contralateral torque. A similar principle would apply for hip efforts in other directions and for right hip efforts. By linking the execution of posture and movement, this model gives a prevalent role to the central command in controlling the contralateral postural adjustments. However, it does not exclude the possible involvement of a feedback mechanism for postural regulation.

Central organization of the coordination between ipsilateral and contralateral torques in subjects with hemiparesis

Despite the use of similar patterns of contralateral torques in hemiparetic and healthy subjects, the magnitudes of the contralateral torques were increased during paretic limb exertions compared with those measured during non-paretic limb exertions or to healthy subject results. It is possible to explain this difference of magnitude by slightly modifying the previously proposed model (Fig. 7B). It is suggested that the gain control located

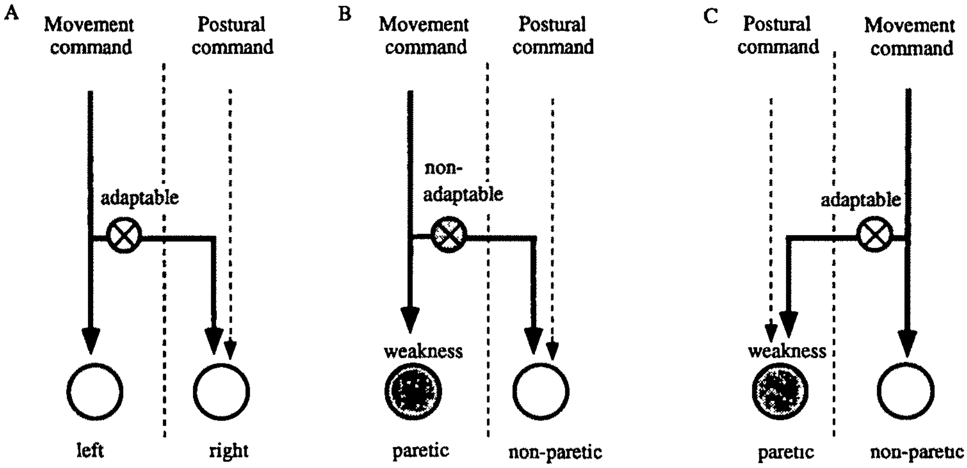

FIG. 7. A, model representing the central organization of the coordination between posture and movement in healthy subjects when using the left limb. This thick lines correspond to the command of movement and the collateral pathways which link the movement command to the postural control. The dotted lines represent the control of postural maintenance. B, modified schema for the coordination between posture and movement in hemiparetic subjects during paretic hip exertions The central command is increased because of muscle weakness and the gain remains fixed generating an increased contralateral torque at the non-paretic limb. C, modified model during non-paretic hip exertions in hemiparetic subjects. The gain adapts to the contralateral muscle weakness and an adequate central command produces an ipsilateral and a contralateral torque of appropriate magnitude.

in the collateral pathways on the non-paretic side is adaptable, but that the gain control located on the paretic side has lost its ability to adapt appropriately, remaining fixed (Fig. 7c). To succeed in the tasks using the paretic limb, because of the paretic muscle weakness, an increased motor command will be necessary. Since the gain control on the paretic side remains fixed, the increased motor command will generate a contralateral torque of an increased magnitude. To succeed in the tasks with the non-paretic limb, assuming that there is no important weakness of the non-paretic muscles, a given command will be sent to the non-paretic limb (Fig. 7c). Since the gain is adaptable, it is possible to increase the effect of this command on the paretic side in order to compensate for the paretic muscle weakness, resulting in an adequate contralateral torque. According to this model, the asymmetry of torques observed during performance with the paretic extremity in hemiparetic subjects is related to the motor weakness of the paretic limb muscles. The weakness is compensated during non-paretic limb effort by the adaptable gain control while it is not during paretic limb efforts, resulting in abnormally high torque of the non-paretic limb. Although this model is simple and probably incomplete, its concept can be useful to interpret results observed in both hemiparetic and healthy subjects and offers a basis for further experimentation.

Clinical application

The question as to whether the increased magnitude of the contralateral torques observed for the hemiparetic subjects during paretic limb efforts is related to, or had an impact on, their performance in functional activities such as gait remains to be evaluated. If such a relationship exists, it would be of clinical interest to quantify it. Moreover, due to the limitations of the present experiment, it would be interesting to test the proposed model with subjects in the standing position. Furthermore, it would be important to evaluate a possible strategy to re-establish the symmetry between the bilateral torques in hemiparetic subjects. One strategy of re-education could include the generation of bilateral symmetrical torques by decreasing the magnitude of the torques exerted on the non-paretic side during paretic hip performance. Such a training protocol could be developed using the dynamometer to giver feedback to the subjects about the magnitude of the bilateral hip torques exerted. The effect of such training on the symmetry of the torques exerted during unilateral paretic limb efforts as well as during functional performances could then be evaluated. Since the results of this study suggest that the weakness of the paretic muscles is responsible for the asymmetrical torques, strengthening the paretic lower limb muscles could be another treatment strategy. This study supports the utilization of certain exercises already used to strengthen or improve the motor control of the paretic lower limb in the hemiparetic population (Brunnstrom, 1970). In fact, similar patterns of contralateral torques were measured in hemiparetic subjects during non-paretic limb exertions and in healthy subjects. A resistance applied to the movements produced at the non-paretic hip in a position where the hips and knees are flexed, would generate adequate patterns of torques at the contralateral paretic lower extremity. The resistance applied to the non-paretic hip could be done either manually or using therapeutic devices. The impact of such exercises on the contralateral paretic limb muscles, on the symmetry of the bilateral torques during unilateral hip efforts and on the functional performances would need to be evaluated.

Conclusion

Similar patterns of contralateral torques were seen in hemiparetic and healthy subjects during static hip efforts. The directions of these contralateral torques were mechanically opposite to the ipsilateral efforts confirming an earlier suggestion from Raïmiste (1911) that the contralateral torques play a role in the stabilization of the pelvis during hip efforts. Furthermore, it seems that these contralateral torques are increased in hemiparetic subjects when performing hip efforts with their paretic limb. Based on a model of postural control, it is suggested that the increased contralateral torques are related to the weakness of the paretic limb muscles and the inability of the gain control on the paretic side to adapt adequately. Since the patterns and magnitude of the contralateral torques in hemiparetic subjects when performing the tasks with their non-paretic limb are, in general, similar to those generated in healthy subjects, we suggest that the contralateral movements observed by Raïmiste (1909, 1911) are caused by weakness of the paretic muscles responsible for the stabilization of the limb. Furthermore, these results support the use of certain exercises for the re-education of paretic hip muscles in hemiparetic subjects which consist of resisting the non-paretic hip movements to strengthen paretic hip muscles.

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DORSAL HORN AND DORSAL COLUMN DYSFUNCTION IN INTRAMEDULLARY CERVICAL CORD TUMOURS

A SOMATOSENSORY EVOKED POTENTIAL STUDY

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SUMMARY

Median and tibial nerves somatosensory evoked potentials (SEPs) were recorded in 20 patients with intramedullary cervical spinal cord tumours. The longitudinal extent of the tumour was determined by magnetic resonance imaging (MRI) and the surgeon's intraoperative observations. Somatosensory evoked potentials to median or tibial nerve stimulation were abnormal in 85% of patients. Three groups were identified on the basis of the median nerve SEP findings: Group I (20% of cases) with normal SEPs responses; Group II (30% of cases) with impaired cervical dorsal horn postsynaptic activity (abnormal N13 potential) but preserved dorsal columns transmission up to the cortex (normal P14 and N20 potentials); Group III (50% of cases) where the dorsal horn activity and dorsal columns transmission were both impaired. The isolated abolition of the N13 potential observed in Group II reflects a functional dissociation between segmental dorsal horn and dorsal columns structures and should prompt myelographic investigations. Postoperative follow-up suggests that absent N13, P14 and N20 potentials before surgery carry an ominous functional prognosis. However, postoperative recovery of the N13 potential was found to occur in a few cases, suggesting that these tumours do not necessarily produce irreversible damage to the cervical dorsal horn neurons. Thus SEPs proved to have a high sensitivity in detecting cervical spinal cord dysfunction in intramedullary tumours, provided that a selective recording of the N13 potential is performed using a cervical-supraglottal derivation.

INTRODUCTION

Intramedullary spinal cord tumours are infrequent; most of the hitherto published series include a limited number of patients and concern either clinical symptomatology, diagnostic difficulties or surgical strategy for their removal (Elsberg, 1925; Slooff *et al.*, 1964). The diagnostic difficulties have been substantially alleviated by the development of neuroimaging techniques including myelography, computerized tomographic (CT) scanning and, more recently, magnetic resonance imaging (MRI). However, clinical signs are often poorly informative for assessing the damage caused by tumoral compression or infiltration to neurons of the dorsal horns (DH) or to fibres of dorsal columns (DC) and pyramidal tracts. In that respect MRI, which proved to be helpful for outlining the limits of the tumour and detecting the presence of a cyst (Di Chiro *et al.*, 1985; Scotti *et al.*, 1987), gives no information concerning the dysfunction of

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spinal cord circuitry. Preoperative detection of this functional damage is helpful since, after some controversies (Wood *et al.*, 1954; Kopelson *et al.*, 1980), the surgical strategy for the treatment of these tumours consists in a complete removal using microsurgical techniques (Greenwood, 1963; Fischer and Tommasi, 1976; Malis, 1978; Fischer and Mansuy, 1980; Cooper and Epstein, 1985), which entails the risk of immediate postoperative accentuation of the neurological deficit. Objective assessment of spinal cord damage is required for addressing the question as to whether this deterioration is related to the involvement of the nervous tissue by the tumour itself or to surgical microtraumatisms, which can be detected by postoperative recordings of somatosensory evoked potentials (SEPs).

Somatosensory evoked potentials permit a non-invasive study of the function of the DC system and DH neurons. The origins of early SEP components elicited by electric stimulation of the upper limb have been clarified by SEP studies using non-cephalic reference montages in normals and patients (Cracco and Cracco, 1976; Kritchevsky and Wiederholt, 1978; Green and MacLeod, 1979; King and Green, 1979; Desmedt and Cheron, 1980; Iragui, 1984; Mauguière, 1987). Concerning the SEP assessment of the DC system it is now widely accepted that (i) Erb's point N9 and scalp P9 potentials reflect action potential volleys in large myelinated fibres of brachial plexus roots (Jones, 1977, 1979; Desmedt *et al.*, 1983); (ii) the cervical N11 and scalp P11 reflect the activity in DC fibres themselves (Desmedt and Cheron, 1980; Anziska and Cracco, 1981; Mauguière, 1983; Iragui, 1984; Suzuki and Mayanagi, 1984); (iii) the scalp P14 far-field potential is produced by the ascending volley in the medial lemniscus (Nakanishi *et al.*, 1978; Anziska and Cracco, 1980; Mauguière *et al.*, 1983*a*; Hashimoto, 1984; Mauguière and Ibáñez, 1985; Yamada *et al.*, 1986; Ibáñez *et al.*, 1989); (iv) the response of the SI cortical area neurons is reflected by the N20 potential recorded in the parietal region contralateral to stimulation (Allison *et al.*, 1980; Desmedt and Cheron, 1981*a*; Mauguière *et al.*, 1983*b*; Deiber *et al.*, 1986). Conduction time in the DC system can also be assessed after lower limb stimulation using tibial nerve SEPs. The cortical parietal activity is reflected by the P39 potential (Desmedt and Cheron, 1983). The segmental postsynaptic response of DH neurons receiving inputs from collateral branches of DC fibres (Dejerine and Thomas, 1896; Ramón y Cajal, 1909) can also be recorded in man after upper limb stimulation as a negative N13 potential using skin electrodes placed at posterior neck (Desmedt and Cheron, 1981*b*; Mauguière, 1983; Emerson *et al.*, 1984; Iragui, 1984). This response is also picked up as a positive potential (spinal P13) at the anterior aspect of the neck (Emerson *et al.*, 1984; Desmedt and Huy, 1984; Mauguière and Ibáñez, 1985) (Fig. 1). The assumption that N13 reflects selectively the activity of DH neurons is mainly based on the two complementary observations that N13 persists, while P14 is absent in lesions at the cervicomedullary junction (Anziska and Cracco, 1980; Mauguière and Ibáñez, 1985; Buchner *et al.*, 1988), and that it is abnormal or absent, with preserved P14 and N20 potentials, in patients with a cervical syrinx and preserved DC function (Emerson and Pedley, 1986; Urasaki *et al.*, 1988; Restuccia and Mauguière, 1991). Similarly the segmental postsynaptic response of DH neurons is recorded at the lumbar level as a negative potential named N22 (Eisen and Odusote, 1980) or N24 (Desmedt and Cheron, 1983). As the cervical N13 this potential can be recorded as a P24 (or P22) positivity using an oesophageal electrode situated anterior to the lumbosacral cord segments at the level of the Th9 vertebra (Desmedt and Cheron, 1983).

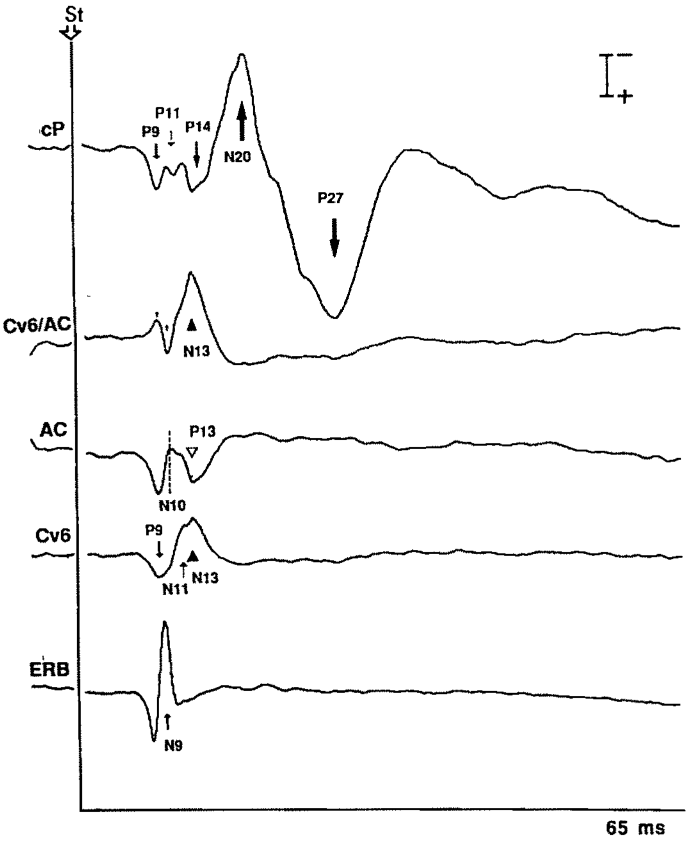

FIG. 1 Normal left median nerve SEPs recorded at: Erb's point (ERB), Cv6 spinous process (Cv6), anterior cervical (AC) and contralateral parietal scalp region (cP) with a non-cephalic reference. The dorsal horn N13 potential is enhanced when recorded with a Cv6 anterior cervical derivation (Cv6/AC). Calibration: 5 μV for the ERB trace, 2.5 μV for the others. St = stimulation.

Previously, SEP findings in intramedullary cervical tumours have been reported in a series of cases including various types of spinal cord lesions (Stöhr *et al.*, 1982; Mauguière *et al.*, 1985; Jabbari *et al.*, 1990). By presenting SEP data obtained in 20 patients with histologically verified intramedullary tumours of the cervical cord (16 ependymomas and four astrocytomas) we aimed at assessing: (i) the validity of SEPs to detect the functional damage to DC fibres and DH neurons with respect to tumoral size and location; (ii) the preoperative prognostic value of SEP findings; (iii) the postoperative SEP evolution with regard to clinical findings.

MATERIAL AND METHODS

Patients

We studied 20 patients aged 15–63 yrs (mean age 40±13 yrs, seven males, 13 females) with intramedullary cervical cord tumours. All patients had median nerve SEP recordings and in 18 of them posterior tibial nerve SEPs were also obtained. Patients were referred to our laboratory with a clinical diagnosis, or suspicion, of spinal cord lesion; all SEP recordings were carried out before surgery and in some cases before that the tumour was evidenced by neuroimaging techniques.

In Table 1 are summarized: (i) the clinical symptoms recorded on the day of SEP recording; (ii) the total extension of the tumour and cyst in the vertical axis of the spinal cord. Magnetic resonance imaging scans were available for 16 patients; in four only myelography or CT were performed. Complete macroscopic microsurgical removal of the tumour was achieved in all cases, including two throughout the entire length of the cord, using a surgical approach previously described in detail by one of us (Fischer and Mansuy, 1980). The longitudinal extent of the tumour was determined by the surgeon.

Somatosensory evoked potential recording methods

Median nerve SEPs. For median nerve SEP recordings, electrodes (impedance below 5 kΩ) were placed at supraclavicular fossa (Erb's point), spinous process of the sixth cervical vertebra (Cv6), and in contralateral and ipsilateral scalp parietal regions. All electrodes were referred to a non-cephalic reference (shoulder contralateral to the stimulated side), except for the Erb's point electrode which was referred to a midfrontal (Fz) electrode in some of the patients.

In order to document better the spinal horn N13 activity, additional derivations were used, i.e. anterior cervical (AC)/shoulder, and Cv6/AC. The AC electrode was placed immediately above the process of the thyroid cartilage. The rationale for the Cv6/AC derivation is that it permits the recording at maximal amplitude of the activity generated by the transverse dipolar source of N13 and spinal P13 potentials (Desmedt and Huy, 1984; Restuccia and Mauguière, 1991). In four patients a circular array of six electrodes regularly spaced around the neck between the Cv6 and AC sites in a plane perpendicular to the neck axis (Fig. 2) was used as an additional technique for recording the N13 and spinal P13 potentials (Desmedt and Huy, 1984; Emerson *et al.*, 1984; Restuccia and Mauguière, 1991), all neck electrodes being referred to the shoulder on the non-stimulated side.

The stimulus consisted in 0.2 ms electric square pulses delivered through skin disc electrodes placed at the wrist (cathode proximal) and was fixed to motor threshold for abductor policis brevis. Interstimulus intervals varied randomly between 425 ms and 525 ms. The bandpass was 1.6 Hz–3.2 kHz (−3 dB at cut-off point, 6 dB down per octave) with an analysis time of 70 ms including a 5 ms prestimulus delay and a bin width of 137 μs (512 samples).

Posterior tibial nerve SEPs. In 18 patients the study of the DC function was completed by the recording of the postsynaptic lumbar N22 (Eisen and Odusote, 1980) and scalp cortical P39 potentials (Jones and Small, 1978; Noël and Desmedt, 1980; Lueders *et al.*, 1981) evoked by electric stimulation of the posterior

TABLE 1 CLINICAL DATA

		Tumour		*Delay (mths)*	*Clinical findings the day of SEP recordings*
Case no.	*Age/sex*	*Type*	*Extension*		
Group I					
1	53/F	E	C2-Th5 (C3-C7)	2	M. left lwl paresis; refl increased in four limbs; T, J, V: normal; P, T°. normal
2	47/F	A	C6-Th9 (Th2-Th7)	48	M: normal, refl increased in four limbs, T, J, V: normal, P, T°. normal
3	54/F	E	C7-Th2 (Th1-Th2)	12	M: normal; refl.: normal; T, J, V: normal; P, T°: normal
4	35/F	E	C4-C5 (C4-C5)	24	M normal, refl.: normal; T, J, V normal; P, T°, normal

		Tumour			
Case no.	*Age/sex*	*Type*	*Extension*	*Delay (mths)*	*Clinical findings the day of SEP recordings*
Group II					
5	20/F	E	C3-Th5 (C4-Th4)	12	M: spastic paraparesis; refl.: reduced tricipital (b), increased in lwls, T, J, V: reduced in lwls; P, T° reduced in lwls
6	48/M	E	C4-Th2 (C6-C8)	6	M: left lwl ma and paresis; refl · increased in lwls; T, J, V reduced joint left lwl; P, T°: normal
7	49/M	E	C6-Th1 (C6-Th1)	12	M: right-hand ma; refl reduced tricipital (b), increased in lwls, T, J, V· normal, P, T°· normal
8	15/F	A	V4-L2 (Th8-Th12)	18	M. paraparesis; refl : abolished in lwls, T, J, V: reduced vibration sense in left lwl, P, T°. normal
9	50/F	E	C2-Th8 (Th2-Th8)	4	M: spastic paraparesis; refl.: increased in four limbs; T, J, V: reduced joint in lwls; P, T°: right lwl hypaesthesia
10	63/F	E	C3-C7 (C3-C7)	12	M: weakness of right hand; refl . increased in right upl and lwl; T, J, V: normal; P, T°: normal
Group III					
11	27/F	E	V4-Th3 (V4-Th3)	2	M. right hemiparesis; refl.: abolished in upls, increased in lwls, T, J, V: reduced vibration sense in lwls, P, T°· normal
12	44/F	E	C1-L1 (Th1-Th3)	?	M: tetraparesis, ma right hand; refl . increased in four limbs; T, J, V (b) anaesth. below Th3, P, T°: lwls hypaesthesia
13	35/M	E	C2-Th4 (C2-Th4)	?	M: ma right upl; refl abolished right upl, increased in lwls; T, J, V reduced right hemibody, P, T° normal

TABLE 1 (*continued*)

Case no	*Age/sex*	*Tumour* *Type*	*Tumour* *Extension*	*Delay (mths)*	*Clinical findings the day of SEP recordings*
14	32/M	E	C2-Th2 (C2-C7)	12	M: left upl paresis; refl : increased in four limbs, T, J, V: reduced in upls, aster. left hand; P, T°: (b) segmental hypaesthesia C3-C6
15	42/F	E	C6-Th10 (C7-Th3)	36	M: paraparesis, refl increased in lwls, T, J, V: reduced vibration sense in lwls, P, T°: right segmental hypaesthesia Th4-Th5
16	51/M	E	C5-C7 (C5-C7)	12	M: left hemiparesis; refl.: abolished in upls, increased in lwls; T, J, V: left upl anaesth , P, T° (b) segmental anaesth. C7
17	17/M	A	Whole spinal cord	6	M. paraplegia; refl : increased in upls, abolished in lwls, T, J, V: reduced in right upl, (b) anaesth. below Th9; P, T°: (b) anaesth. below Th9
18	43/M	E	C2-Th1 (C3-C7)	18	M: left hemiparesis; refl.: increased in four limbs; T, J, V: anaesth. in upls, left ataxic hand; P, T°: hypaesthesia in four limbs
19	28/F	A	V4-C6 (V4-C6)	20	M: right hemiplegia, ma at right upl and lwl; refl. reduced in upls, increased in lwls; T, J, V anaesth. four limbs and trunk, (b) ataxic hands; P, T°: anaesth. four limbs and trunk
20	49/F	E	C4 (C4)	12	M: tetraparesis, ma both hands, refl.: reduced in upls, increased in lwls, T, J, V: anaesth. four limbs and trunk; P, T°: anaesth. four limbs and trunk

A = astrocytoma; anaesth. = anaesthesia, aster. = astereognosis; (b) = bilateral; delay = delay between clinical onset and diagnosis; ? = delay unknown, E = ependymoma, extension = total extension of tumour and cyst. The extent of the tumoral mass itself is indicated between the parentheses lwl(s) = lower limb(s); M = motor signs; ma = muscular atrophy; refl. = reflexes; P, T° = pain and temperature, T, J, V = touch, joint, vibration sense; upl(s) = upper limb(s).

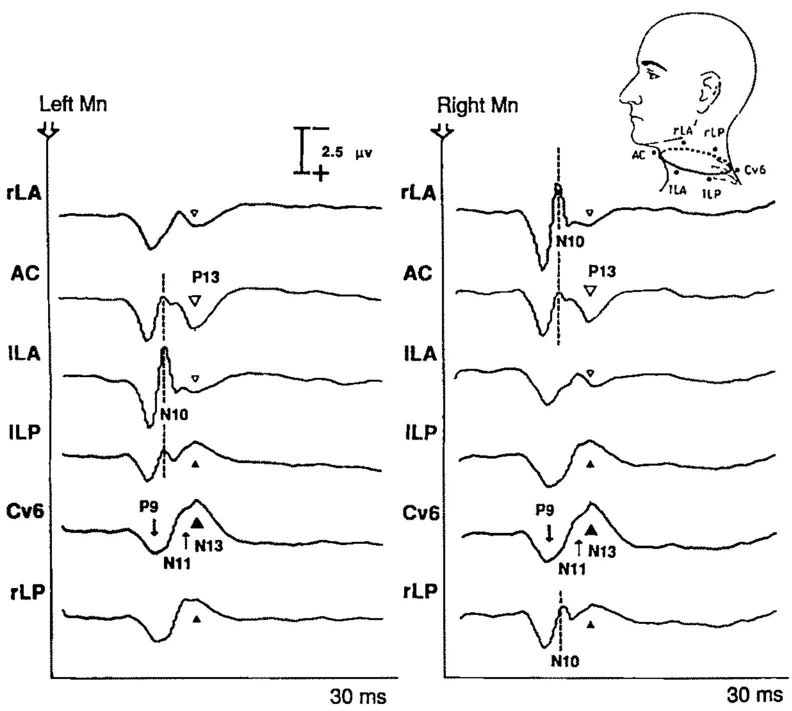

FIG. 2. Normal left and right median (Mn) nerve SEPs recorded with a circular array of cervical electrodes connected to a non-cephalic reference. The N13 potential is recorded over the cervical posterior regions with maximal amplitude at Cv6, whereas the P13 potential appears at the cervical anterior regions with maximal amplitude at AC. A 'root' potential N10 is recorded ipsilaterally to the side stimulated. Recording sites: right lateral anterior (rLA), left lateral anterior (lLA), right lateral posterior (rLP), left lateral posterior (lLP), Cv6 and AC

tibial nerve at the ankle. Tibial nerve SEPs were recorded using electrodes placed at L1 spinous process and at the vertex on the scalp (4 cm behind the international Cz position). Oesophageal recordings (Desmedt and Cheron, 1983) were not used in this study. The lumbar and scalp electrodes were referred respectively to the knee and ear lobe contralateral to stimulation. The tibial nerve was stimulated at the ankle at motor threshold. Interstimulus interval, filter bandpass and stimulus were the same as for median nerve SEPs. The analysis time was of 105 ms including a 5 ms delay with a bin width of 103 µs (1024 samples).

Somatosensory evoked potential analysis

Normative data obtained in normal subjects after stimulation of median nerve (25 subjects) and posterior tibial nerve (24 subjects) are given in Table 2. In patients, latency values outside the mean ±2.5 SD range of control population were considered abnormal. Somatosensory evoked potential components were identified visually and their peaking latencies were automatically measured by the computer between two cursors. The scalp far-field P11 potential was not studied for it is known that it can be missing in about 30% of normal subjects (Mauguière, 1983). The P9-P14 interval was used to evaluate conduction in proximal roots and DC, up to the cervico-medullary junction. Amplitude values given in Table 2 were calculated from base-line to peak; the amplitude of the contralateral parietal N20 potential was measured after subtraction of the ipsilateral parietal response in order to cancel all subcortical SEP components (Mauguière and Desmedt, 1988; Ibáñez *et al.*, 1989). Absolute amplitude values cannot be used as normative data due to their poor Gaussian distribution. The following amplitude parameters were calculated for N13, P14 and N20 potentials: (i) the N13/P9 amplitude ratio and the logarithmic values of this ratio (using the Cv6/AC derivation), as recently proposed in our laboratory (Restuccia and Mauguière, 1991); (ii) the P9/P14 amplitude ratio

TABLE 2 NORMATIVE DATA

Median nerve (mean age 35±11.5 yrs, 25 subjects; 50 median nerves)

	N13/P9 ratio	*N13/P9 (log values)*	*P9/P14 ratio*	*N20 percentage asymmetry side to side*
Amplitudes (μv)				
Mean	2.93	0.43	0.64	21.43
SD	1.17	0.13	0.22	10.42

	N9	*N13*	*P9*	*P14*	*N20*	*Interpeak P9-P14*
Latencies (ms)						
Mean	10.45	13.65	9.69	14.5	19.49	4.85
SD	0.85	0.95	0.88	0.95	1.22	0.56

Posterior tibial nerve (mean age 37±9.1 yrs; 24 subjects; 48 tibial nerves)

	N22	*P39*
Amplitudes (μv)		
Mean	1.38	2.92
SD	0.7	1.36

	N22	*P39*	*CCT*
Latencies (ms)			
Mean	21.23	39.54	18.53
SD	1.43	1.91	1.86

(García-Larrea and Mauguière, 1988); (iii) the percentage of amplitude difference of the N20 potential between the two sides (Mauguière and Desmedt, 1988). In some subjects the potential which we labelled above P14 can be made of two subcomponents, namely P13 and P14. In such cases the amplitude of the larger of the two peaks was used when calculating the P9/P14 ratio as reported earlier (García-Larrea and Mauguière, 1988). A selective loss of the P14 potential has been reported in lesions of the medulla oblongata (Wang *et al.*, 1982; Delestre *et al.*, 1986) in favour of separate generators for P13 and P14 in the lower brainstem. Moreover, the question whether the P13 potential might reflect the activity of presynpatic terminals in the nucleus cuneatus has received controversial answers (Møller *et al.*, 1986; Ibáñez *et al*, 1989; Nardone and Schieppati, 1989; Morioka *et al.*, 1991) The important point for this study is that both P13 and P14 have a brainstem origin. Therefore we used the label 'P14' in the few patients in whom these two components were identifiable. This has the advantage of avoiding any confusion between brainstem and spinal P13 potentials (*see above*).

Clinical and SEP follow-up

Somatosensory evoked potential recording and clinical examination were repeated postoperatively in 16 of the 20 patients. The delay between surgery and postoperative SEP recordings ranged from 10 d to 2 yrs.

RESULTS

Preoperative median nerve SEPs findings

Preoperative SEP findings are detailed in Table 3. On the basis of median nerve SEP data obtained on the most affected side it was possible to individualize three separate groups of patients.

TABLE 3 SOMATOSENSORY EVOKED POTENTIAL PREOPERATIVE DATA

	Median nerve					*Tibial nerve*	
Case no.	*Side*	*N9*	*N13*	*P14*	*N20*	*N22*	*P39*
Group I							
1	R	N	N	N	N	N	N
	L	N	N	N	N	N	Abs
2	R	N	N	N	N	N	N
	L	N	N	N	N	N	N
3	R	N	N	N	N	N	N
	L	N	N	N	N	N	N
4	R	N	N	N	N	N	N
	L	N	N	N	N	N	N
Group II							
5	R	N	Red	N	N	N	D
	L	N	Abs	N	N	N	D
6	R	N	Abs	N	N	N	D
	L	N	Abs	N	N	N	D
7	R	N	Abs	N	N	N	N
	L	N	Abs	N	N	N	D
8	R	N	Abs	N	N	N	N
	L	N	N	N	N	N	N
9	R	N	N	N	N	N	Abs
	L	N	Abs	N	N	N	D
10	R	N	Abs	N	N	N	Abs
	L	N	Abs	N	N	N	Abs
Group III							
11	R	N	Abs	D*	D**	N	N
	L	N	Abs	N	N	N	D
12	R	N	Abs	N	N	N	Abs
	L	N	Abs	D*	D**	N	Abs
13	R	N	Abs	D*	D**	N	D
	L	N	N	N	N	N	N
14	R	N	Abs	D*	D	N	N
	L	N	Abs	D*	D	N	N
15	R	N	Red	D*	D	N	N
	L	N	Red	D*	D	N	N
16	R	N	Abs	N	N	–	–
	L	N	Abs	Abs	Abs	–	–
17	R	N	Abs	Abs	Abs	Abs	Abs
	L	N	N	N	N	Abs	Abs
18	R	N	Abs	Abs	Abs	N	N
	L	N	Abs	Abs	Abs	N	N
19	R	N	Abs	Abs	Abs	–	–
	L	N	Abs	Abs	Abs	–	–
20	R	N	Red	Abs	Abs	N	Abs
	L	N	Red	Abs	Abs	N	Abs

– = not done, * = abnormal P9/P14 ratio with reduced P14 potential, ** = side to side amplitude difference greater than 50% for the N20 potential; Abs = absent; D = delayed, L = left, N = normal; Red = reduced N13/P9 amplitude ratio, R = right.

Group I. Normal SEPs. Four patients (Cases 1, 2, 3, 4) presented with normal median nerve SEPs on both sides in favour of preserved DH activity and DC conduction in the cervical spinal cord.

Group II. Abnormal N13 response with normal P9-P14 interval and parietal N20. An abnormal response of DH neurons, coexisting with preserved conduction in the DC system, was detected in six patients in whom abolition or amplitude reduction of the N13 potential represented the only abnormal SEP finding. This abnormality was bilateral in four patients (Cases 5, 6, 7, 10) and unilateral in two (Cases 8, 9). Traces obtained using a cervical array of six electrodes in one of these patients are illustrated in Fig. 3. They show that the brachial plexus P9 potential was recorded at Cv6 and AC recording sites; it was followed, at the AC site and at latero-cervical site ipsilateral to stimulation, by the proximal root N10 potential (Desmedt and Huy, 1984; Emerson *et al.*, 1984). After right median nerve stimulation there was no cervical N13 or spinal P13 potentials at any of the recording sites. In all of these six patients the abnormal DH response contrasted with normal DC conduction as assessed by normal brainstem P14, cortical N20 and P9-P14 interval.

Group III. Abnormal N13, P14 and N20 potentials. A dysfunction of both DH neurons and DC fibres was evidenced by median nerve SEPs in 10 patients in whom absent or reduced N13 potential was observed in conjunction with (i) a prolonged P9-P14 interval or absent P14 potential; (ii) a delayed or absent N20 potential. This SEP pattern was bilateral in five patients. Persistence of only the Erb's point N9 and the cervical and scalp P9 brachial plexus potentials with absent P14 and N20 represented the most conspicuous SEP abnormality in five patients (indicating interruption of input transmission at the entry zone of cervical roots); it was bilateral in three patients (Fig. 4) and unilateral in two (Fig. 5).

In three patients of this group, SEPs of the less affected side showed normal DC conduction reflected by normal P9-P14 interval and N20 potential (Fig. 6).

Preoperative tibial nerve SEPs

The lumbar N22 potential was normal in all patients except one (Case 17) in whom this potential was absent bilaterally. The cortical P39 potential was found delayed or absent at least on one side in 11 of 18 patients in whom tibial nerve SEPs were recorded. This dysfunction of the lower limb DC fibres evidenced by tibial nerve SEPs contrasted with the normal conduction in upper limb DC fibres on the same side in patients with normal median nerve SEPs (Table 3, Fig. 7). This was observed, at least in one side, in one of the patients of Group I (Case 1), in five patients of Group II (Cases 5, 6, 7, 9, 10) and in three patients of Group III (Cases 11, 12, 17). Thus combined median and tibial nerve SEPs confirmed that damage to DC can affect fibres coming from lower limbs selectively. This was shown in one patient with an intramedullary tumour located at the cervical cord level (Case 10).

Correlation between preoperative clinical and SEP data

Correlations between median and tibial nerve SEPs and clinical findings in the corresponding territories are summarized in Table 4. The total numbers of SEPs referred to in this table are of 40 and 36 for median nerve and tibial nerve SEPs, respectively.

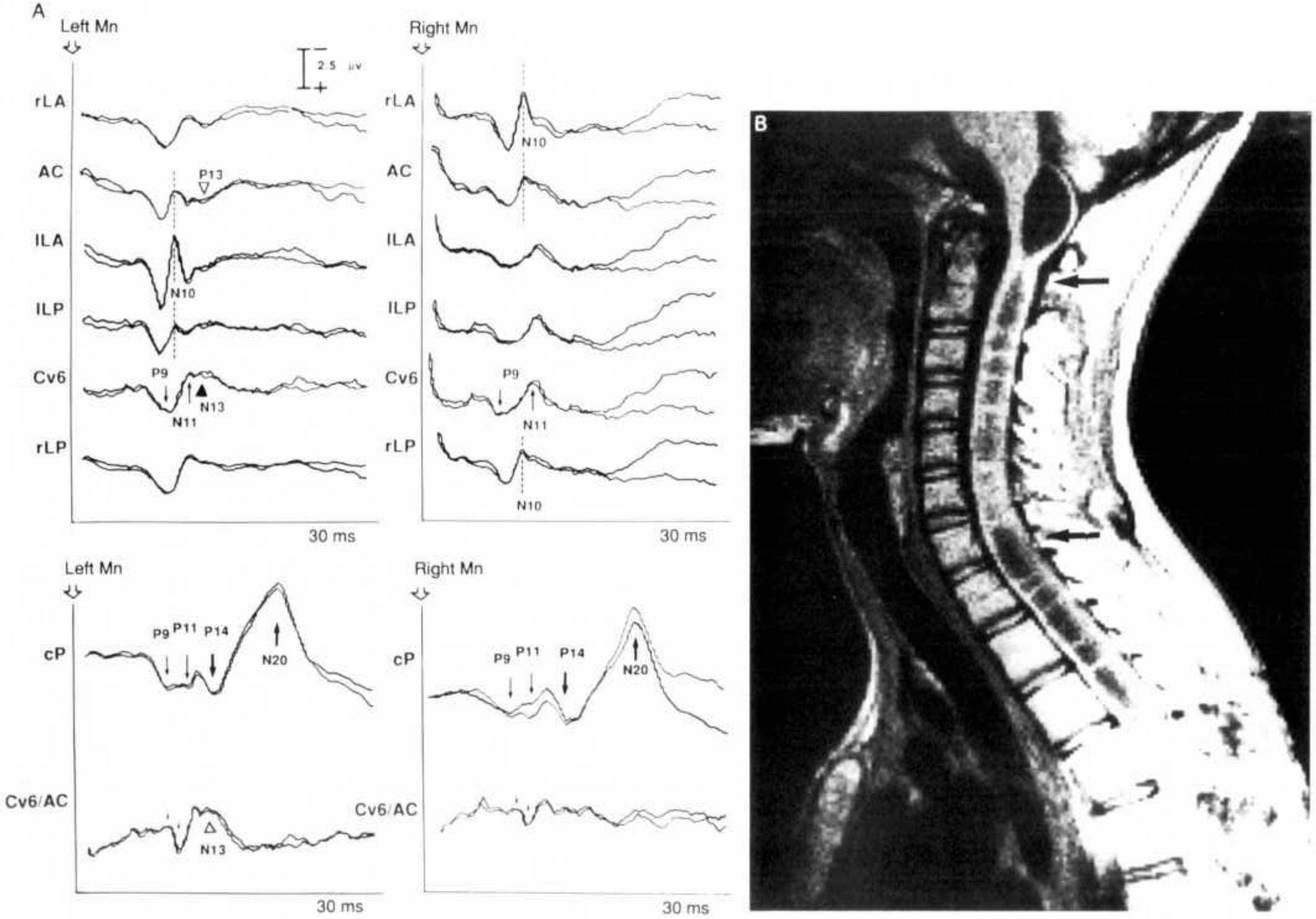

FIG. 3. *Case 8*: astrocytoma (V4-L2). A (*top*), traces recorded with the same circular array of cervical electrodes as in Fig. 2. The interpeak latency N10-N11 is similar for both median nerves but the N13 and the P13 potentials are absent after right median nerve stimulation. A (*bottom*), the Cv6/AC derivation shows a clear asymmetry with absence of the N13 potential after right median nerve stimulation, but the far-field P9, P11, P14 and cortical parietal N20 are normal for both median nerves. B, MRI sagittal view of the cervical and thoracic spinal cord. The arrows delimit the cervical extension of the cyst (C2 to C7 vertebrae).

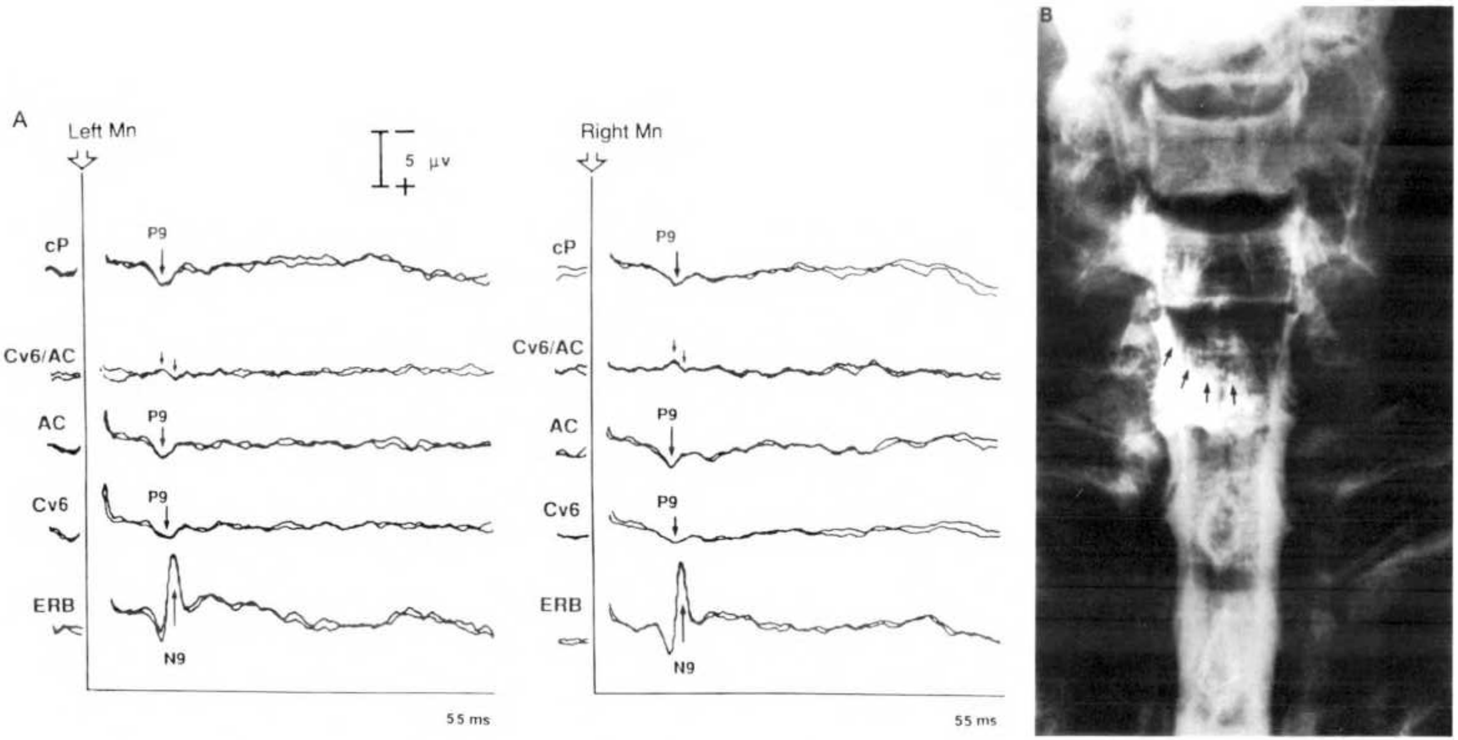

FIG. 4. *Case 18*: ependymoma (C2-Th1). Bilateral interrupted input at spinal cervical cord entry. A, only brachial plexus near-field N9 and far-field P9 are recorded after left and right median nerve stimulation. The dorsal horn N13 and P13 potentials as well as the dorsal column P11, brainstem P14 and cortical N20 potentials are absent bilaterally. B, myelography showing a stop in the contrast at the C7 vertebra. The black arrows indicate the lower pole of the tumour.

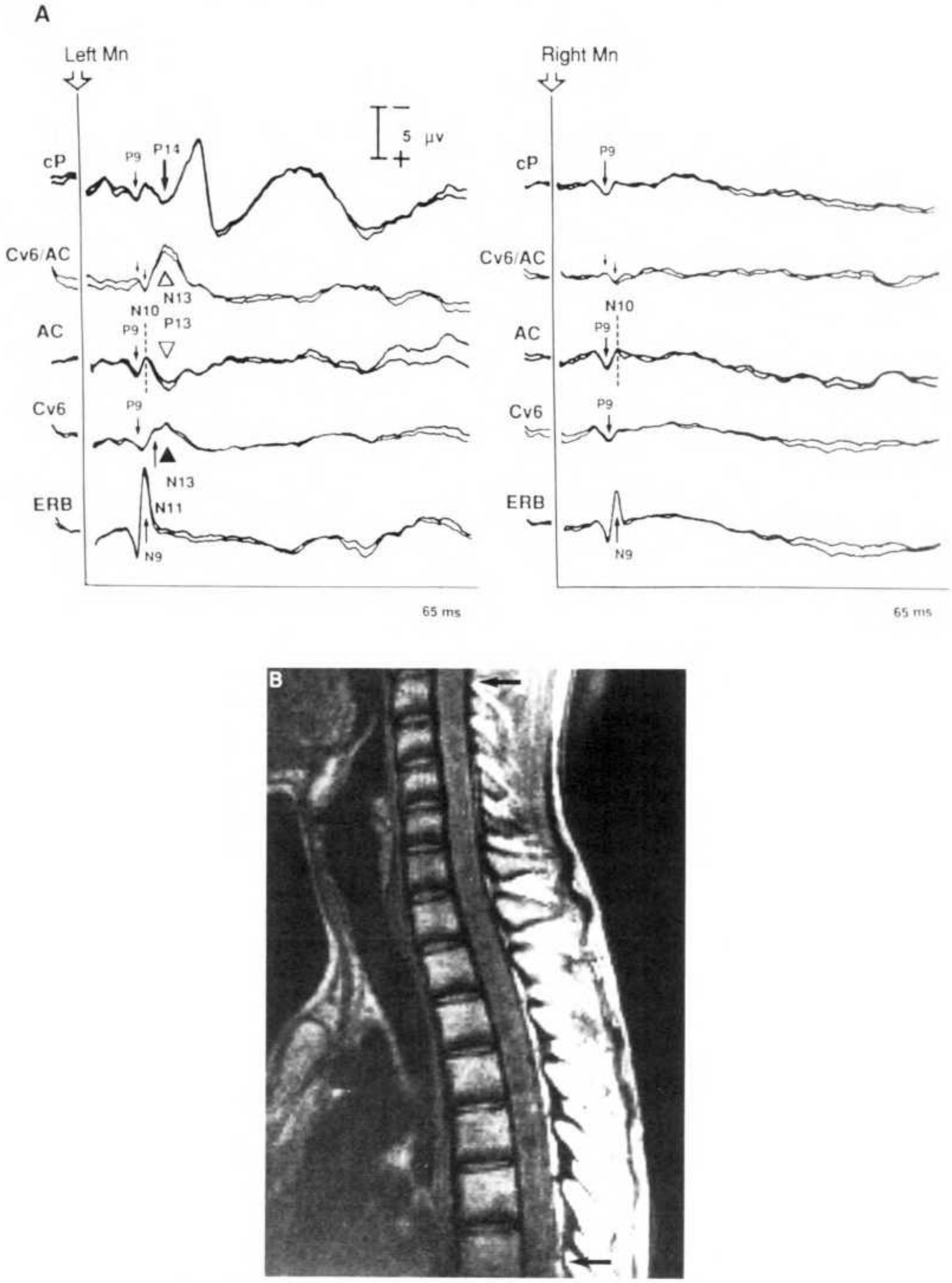

FIG. 5. *Case 17*: astrocytoma (whole spinal cord). Unilateral interruption of input transmission at spinal cervical cord entry. A, absence of all SEP components except the brachial plexus N9 and P9, after right median nerve stimulation. B, MRI sagittal view of the cervical and thoracic spinal cord showing a regular enlargement of the cord from C2 vertebra to Th6 vertebra (black arrows).

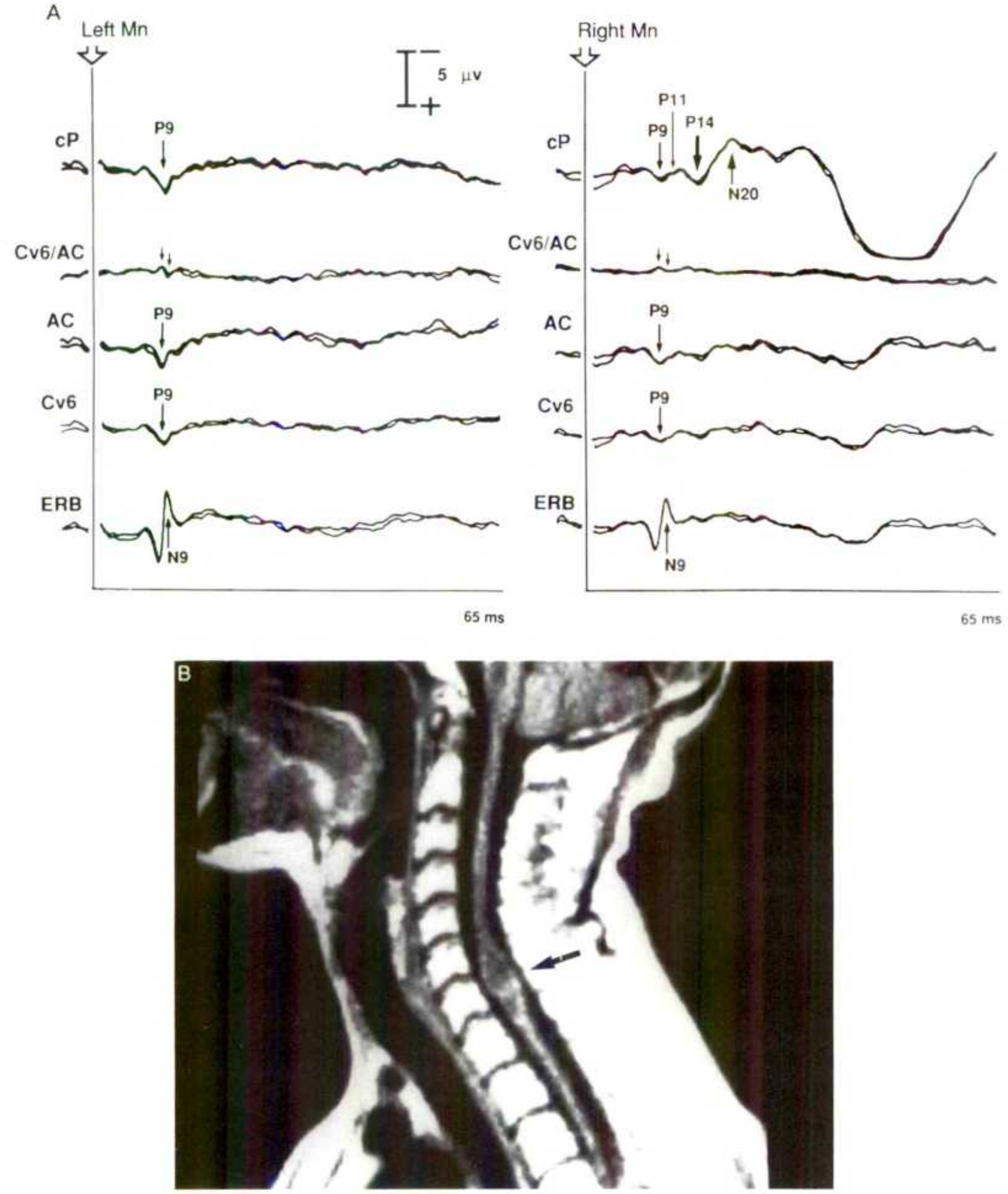

FIG. 6. *Case 16*: ependymoma (C5-C7). A (right median nerve), isolated abolition of the cervical N13 and P13 potentials at Cv6 and AC recording sites with absent N13 at the Cv6/AC trace. A (left median nerve), absence of all SEP components except the brachial plexus N9 and P9 potentials. B, MRI sagittal view showing the tumour with maximal width at the level of the C7 vertebra (black arrow).

When all median nerve SEP components were normal, there was no clinical evidence of impaired tactile, joint, vibration, pain and temperature sensations in the corresponding upper limb; moreover, biceps and brachioradialis reflexes were normal. Tactile, joint

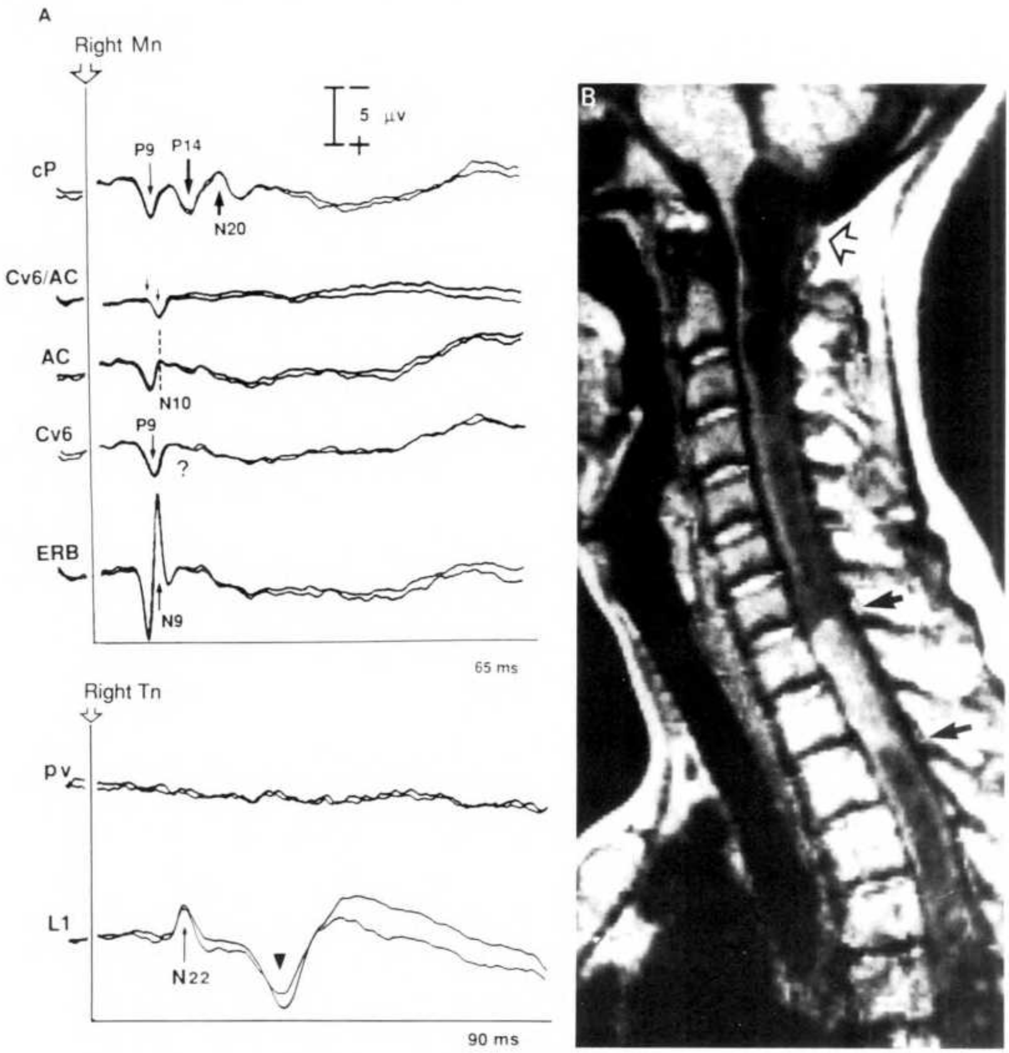

FIG. 7. *Case 12*: ependymoma (C1-L1). A (*top*), the N13 potential is absent after right median nerve stimulation. The Cv6/AC derivation detects this abolition clearly, although a doubt could persist in the Cv6 non-cephalic traces. A (*bottom*), at the scalp posterior vertex (pv) recording site, the cortical parietal P39 is absent after right tibial nerve stimulation, with normal N22 recorded at the lumbar spinal process (L1). The black triangle shows a myogenic potential recorded in this spastic patient. B, MRI sagittal view of the cervical and thoracic cord showing the tumoral mass at the level of Th1 to Th3 vertebrae (black arrows) topped by a cervical cyst extending up to medulla oblongata (open arrow).

and vibration sensations were also preserved when only the cervical N13 potential was abnormal after median nerve stimulation. A segmental loss of pain and temperature sensation in the upper limbs was found in two patients only (Cases 14, 16); in both of them it was bilateral and the N13 potential was also bilaterally absent. Sub-lesional loss of pain and temperature sensation was observed in six of the 40 upper limbs; the N13 potential was abnormal in all cases after stimulation of the median nerve on the

TABLE 4. SENSATION, REFLEXES AND SEPs DATA

	Pain and T°		*Touch sense*		*Joint sense*		*Vibration sense*		*Reflexes*	
	Normal	*Abnormal*	*Normal*	*Abnormal*	*Normal*	*Abnormal*	*Normal*	*Abnormal*	*Normal or increased*	*Absent or reduced*
Median nerve SEPs										
Normal N13, P14, N20 (n = 12)	12	0	12	0	12	0	12	0	12	0
Abnormal or absent N13; normal P14, N20 (n = 13)	12	1	13	0	13	0	13	0	7	6
Abnormal or absent N13, P14, N20 (n = 15)	6	9	4	11	4	11	4	11	8	7
Tibial nerve SEPs										
Normal P39 (n = 18)	16	2	18	0	18	0	14	4	16	2
Absent or delayed P39 (n = 18)	9	9	9	9	6	12	8	10	16	2

side of anaesthesia. Moreover, biceps and brachioradialis reflexes could not be obtained, or were clearly reduced when compared with the other side, in 13 upper limbs. Isolated loss or reduction of N13 was statistically correlated only with impaired reflexes in upper limbs (corrected $\chi^2 = 4.97$, $P < 0.05$).

Thus SEPs disclosed abnormal DH responses (as assessed by reduced or absent N13) which were not detected by clinical examination of pain and temperature sensations or reflexes in 18 and 15 upper limbs. Similarly median and tibial nerve SEPs disclosed an infraclinical dysfunction of DC fibres in four of 15 upper limbs and six of 18 lower limbs where tactile, joint and vibration sensations were all normal.

Duration of the disease, lesion site and median nerve SEP findings

The duration of the disease and the size of the lesion (number of metameric levels) were not significantly different (*t* test $P > 0.05$) between the three groups of patients individualized on the basis of median nerve SEP findings.

Postoperative SEPs and clinical evolution

Somatosensory evoked potentials and clinical data recorded after surgery in 16 of our 20 patients are detailed in Table 5. In seven patients (Cases 2, 3, 6, 10, 14, 16, 18) SEPs to median or posterior tibial nerve were unchanged. No motor or sensitive clinical changes were observed in these patients except in three who developed a segmental pain and temperature hypaesthesia in the postoperative period (Cases 2, 3, 18).

In six patients (Cases 1, 4, 7, 8, 11, 13) either median nerve or tibial nerve SEPs, or both, worsened postoperatively. In all of these patients sensory performances deteriorated. Four of these patients (Cases 1, 4, 11, 13) presented a segmental loss of pain and temperature sensation in upper limbs, which had not been detected before surgery. In one of the patients (Case 8) with a tumoral cyst at the cervical level, the N13 potential recovered after surgery while the tibial nerve P39 potential, which was normal before surgery, disappeared bilaterally after the removal of the tumour. In three patients the motor deficit increased postoperatively; all of them, however, were still able to walk.

In three patients (Cases 5, 15, 20) SEPs improved after surgery. As illustrated in Figs 8 and 9, this improvement mainly concerned the N13 potential, which progressively

TABLE 5. CLINICAL AND SEPs POSTOPERATIVE DATA

Case no.	*Clinical findings at postoperative SEP recordings*	*Delay*	*Side*	*Median nerve*				*Tibial nerve*	
				N9	*N13*	*P14*	*N20*	*N22*	*P39*
Group I									
1	M: paraparesis; refl.: abolished in left upl, increased in lwls; T, J, V: reduced below C4; P, T°: left hypaesthesia below C6	10 d	R	N	Abs	D*	D	N	Abs
			L	N	Abs	D*	D	N	Abs
2	M: normal; refl.: increased in lwls; T, J, V: normal; P, T°: right segmental anaesth. Th5-Th10	17 d	R	N	N	N	N	N	N
			L	N	N	N	N	N	N
3	M: normal; refl.: increased in lwls; T, J, V: normal; P, T°: segmental hypaesthesia Th1-Th4 (b)	16 mths	R	N	N	N	N	N	N
			L	N	N	N	N	N	N
4	M: normal; refl.: normal; T, J, V: normal; P, T°: right segmental hypaesthesia C8-Th1	4 mths	R	N	Abs	D*	D**	N	N
			L	N	N	N	N	N	N
Group II									
5	M: normal; refl.: normal; T, J, V: reduced in left lwl; P, T°: normal	12 mths	R	N	N	N	N	N	N
			L	N	N	N	N	N	N
6	M: unchanged; refl.: abolished in upls; T, J, V: reduced lwls; P, T°: normal	2 mths	R	N	Abs	N	N	N	D
			L	N	Abs	N	N	N	D
7	M: right-hand ma and right hemiparesis; refl.: unchanged; T, J, V: reduced below Th1 (b); P, T°: normal	3 mths	R	N	Abs	N	N	N	N
			L	N	Abs	N	N	N	Abs
8	M: unchanged. refl.: abolished in lwls; T, J, V: reduced in lwls; P, T°: normal	6 mths	R	N	N	N	N	N	Abs
			L	N	N	N	N	N	Abs
9	Not done		–	–	–	–	–	–	–
10	M: unchanged; refl.: unchanged; T, J, V: normal; P, T°: normal	2 mths	R	N	Abs	N	N	N	Abs
			L	N	Abs	N	N	N	Abs
Group III									
11	M: right hemiplegia, left lwl paresis; refl.: unchanged; T, J, V: reduced right upl and lwls; P, T°: right segmental hypaesthesia C4-Th6	1 yr	R	N	Abs	Abs	Abs	N	Abs
			L	N	Abs	D*	D	N	Abs
12	Not done		–	–	–	–	–	–	–
13	M: unchanged; refl.: unchanged; T, J, V: reduced right hemibody; P, T°: hypaesthesia right upl	20 d	R	N	Abs	Abs	Abs	N	Abs
			L	N	N	N	N	N	N
14	M: unchanged; refl.: unchanged; T, J, V: reduced in upls; aster. (b); P, T°: unchanged	15 d	R	N	Abs	D*	D	N	N
			L	N	Abs	D*	D	N	N
15	M: unchanged; refl.: increased in four limbs; T, J, V: unchanged; P, T°: unchanged	2 yrs	R	N	N	D*	D	N	N
			L	N	N	D*	D	N	N
16	M: left hemiplegia; refl.: reduced in upls, increased in lwls; T, J, V: unchanged; P, T°: unchanged	10 mths	R	N	Abs	N	N	–	–
			L	N	Abs	Abs	Abs	–	–

TABLE 5 (*continued*)

Case no.	*Clinical findings at postoperative SEP recordings*	*Delay*	*Side*	*Median nerve*				*Tibial nerve*	
				N9	*N13*	*P14*	*N20*	*N22*	*P39*
17	Not done		–	–	–	–	–	–	–
18	M: tetraparesis; refl.: abolished in upls, increased in lwls; T, J, V: anaesth. in upls, (b) ataxic hand; P, T°: (b) segmental anaesth. C4-Th1	2 yrs	R L	N N	Abs Abs	Abs Abs	Abs Abs	N N	N N
19	Not done		–	–	–	–	–	–	–
20	M: partial recovering of tetraparesis; refl.: increased in lwls; T, J, V: unchanged; P, T°: unchanged	2 mths	R L	N N	N N	Abs Abs	Abs Abs	N N	Abs Abs

– = not done; * = abnormal P9/P14 ratio with reduced P14 potential; ** = side to side amplitude difference greater than 50% for the N20 potential; Abs = absent; anaesth. = anaesthesia; aster. = astereognosis; (b) = bilateral; D = delayed; delay = delay between surgery and postoperative SEPs; lwl(s) = lower limb(s); M = motor signs; ma = muscular atrophy; N = normal; R, L = right, left side; refl. = reflexes; P, T° = pain and temperature, T, J, V = touch, joint, vibration sense; upl(s) = upper limb(s).

increased in amplitude and decreased in latency. This evolution was paralleled by a recovery of biceps and brachioradialis tendon reflexes when they had been lost or reduced before surgery. In Case 3, tibial nerve SEPs also recovered progressively (Fig. 9).

DISCUSSION

Magnetic resonance imaging and SEPs in the preoperative assessment of cervical cord tumours

Several studies have already emphasized the utility of SEPs to detect a functional damage to the DC system in various pathologies (Jones *et al.*, 1980, 1982; Mauguière *et al.*, 1985; Garcia-Larrea and Mauguière, 1988; Jabbari *et al.*, 1990; Turano *et al.*, 1991). This study shows that median nerve SEPs can also detect abnormal segmental response of DH neurons in 80% of patients with intramedullary cervical cord tumours. Though diagnosis of intramedullary tumours relies exclusively on neuroimaging techniques, magnetic resonance images do not provide any information on spinal cord function. One of the major conclusions from this study is that there is no simple relation between the degree of SEP abnormalities and the longitudinal extent of the tumour, as assessed by preoperative MRI and the surgeon's observation. Comparative study between the axial extension of the tumour in the spinal cord and SEPs was not possible since axial MRI images were not available.

Clinical utility of preoperative SEPs: spinal segmental SEP versus central conduction time abnormalities

The initial symptoms of the cervical cord tumours are often poorly evocative, consisting in pain at the neck or back or limb paraesthesiae. This results in a considerable delay between clinical onset and diagnosis which was of 1 yr or more in 13 of our 20 patients (*see* Table 1). When referred to our laboratory only three patients had normal median

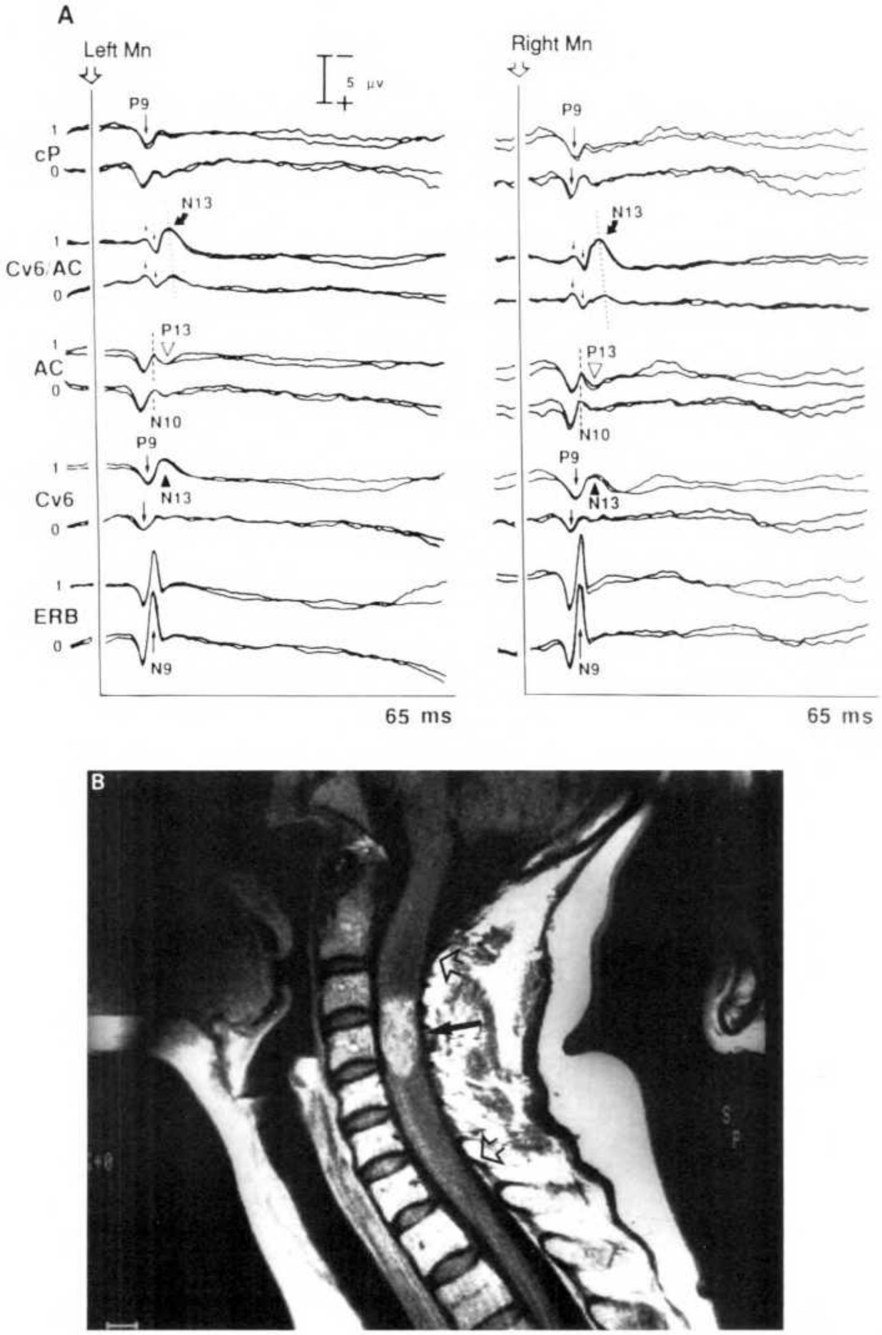

FIG. 8. *Case 20*: ependymoma (C4). A, before surgery (0), N13 is reduced in amplitude in Cv6/AC traces whereas far-field brainstem P14 and cortical parietal N20 potential are absent. A, after surgery (1), N13 recovers in latency and amplitude but P14 and N20 potentials remain absent. B, MRI sagittal view of the cervical cord showing the tumoral mass at the level of the C4 vertebra (black arrow), as well as a global enlargement of the cervical cord above and below the tumour (open arrows).

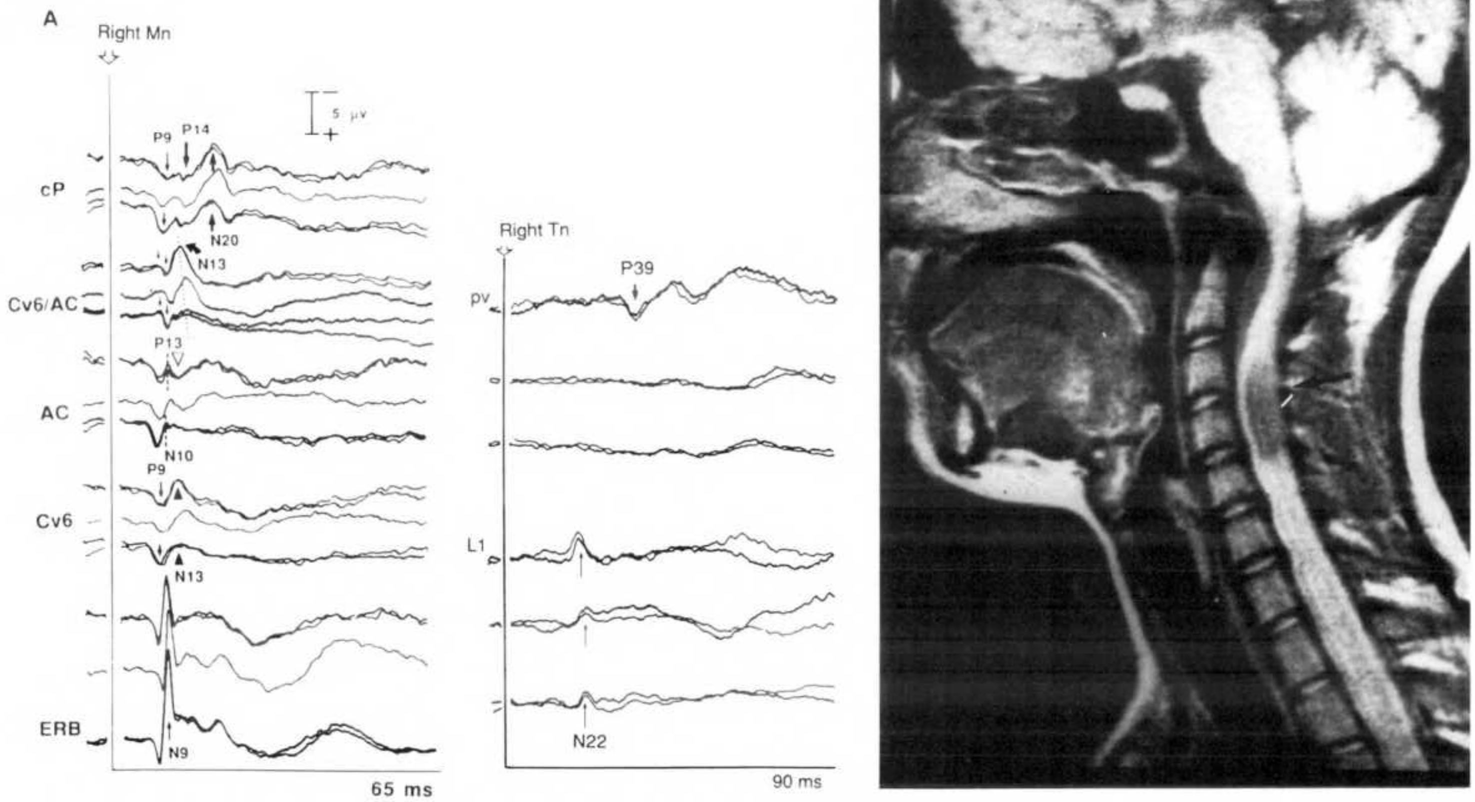

FIG. 9. *Case 5*: ependymoma (C3-Th5). A (*left*), right median nerve SEPs, obtained for each derivation, before surgery (lower traces), 2 wks (middle traces) and 12 mths (upper traces) after surgery. Before surgery N13 was reduced in amplitude whereas P14 and N20 were normal. After surgery N13 recovered progressively in latency and amplitude and the P14 and N20 potentials did not show any change. A (*right*), normal N22 potential recorded at lumbar spinal process (L1) before and after surgery (same presentation as for median nerve SEPs). The cortical parietal P39 potential which was absent before surgery, recovered 12 mths after surgery. B, MRI sagittal view of the spinal cord showing a cyst at the level of C3 and C4 vertebrae (black arrow). Note the enlargement of the spinal cord below the cyst produced by a tumoral mass extending down to Th4 vertebra.

and tibial nerve SEP responses; sensations were normal in the three of them and the only abnormal clinical finding was an increase of tendon reflexes in the four limbs in one.

Although none of the SEP abnormalities which we observed can be considered as specific or intramedullary tumours, an important finding was that, in all patients with abnormal SEPs, the cervical N13 potential was reduced or absent, at least one side, after stimulation of median nerves. This segmental SEP abnormality reflects a reduced response of DH neurons at the cervical level. It should prompt myelographic investigations, in particular, when observed in patients whose SEPs do not show slow conduction in DC fibres, i.e. when the P9-P14 interval and the N20 potential are normal as observed in our six patients of Group II. When combined with abnormal conduction times in DC fibres or with absent P14 and N20 potentials, an abnormal N13 potential can be recorded in any type of pathology affecting the lower cervical cord, including multiple sclerosis. Conversely, isolated loss of N13, which has also been reported in syringomyelia (Urasaki *et al.*, 1988; Restuccia and Mauguière, 1991), is unlikely to occur in demyelinating diseases (Garcia-Larrea and Mauguière, 1988; Turano *et al.*, 1991), or in spinal cord compression, the effect of which on cord function is primarily a slowing of conduction in DC fibres. Anterior compression of the cord interfering with blood supply by the anterior spinal artery could, in theory, affect selectively the response of DH neurons to afferent inputs from the periphery. This hypothesis was recently supported by data from Restuccia *et al.* (1992) showing subclinical amplitude reduction of the cervical N13 in patients with anterior cord compression due to cervicarthrosis, and preserved conduction times in the DCs. Our study also shows that, for intramedullary tumours, correlations between SEPs and clinical findings are not as simple as for cervical syringomyelic cavities (Restuccia and Mauguière, 1991) since the isolated loss of N13 observed in 13 median nerve SEPs in this series was associated only once with a segmental hypaesthesia for pain and temperature.

Conduction in DC fibres, as evaluated by combining median and tibial nerve SEPs, was abnormal in 16 of the 17 patients with abnormal SEPs; however, SEP signs of abnormal conduction in DC fibres do not give any information on the level of segmental cord dysfunction. In six patients this DC conduction slowing was detectable only on tibial nerve SEP recordings. A greater sensitivity of gracile tract fibres to cervical cord compression has been proposed to explain isolated tibial nerve SEP abnormalities in various types of cervical cord compression, including cervicarthrosis (Noël and Desmedt, 1980; Yu and Jones, 1985). In intramedullary tumours an alternative explanation is that the lesion can infiltrate the gracile but not the cuneate tracts. Moreover, this study shows that conduction can be selectively impaired in cuneate tract fibres, as observed in three patients whose N20 was bilaterally absent with normal P39 potentials, thus suggesting that some of these tumours can infiltrate only the outer part of the DC on both sides.

Somatosensory evoked potentials and postoperative outcome

A return to normal of median nerve SEPs was never observed after surgery in Group III patients whose N13, P14 and N20 potentials were abnormal before surgery. Considering that none of these patients improved clinically after surgery, this preoperative SEP pattern carries an ominous functional prognosis.

Recovery of DC conduction is not frequent since it was observed in only one patient after tibial nerve stimulation (Fig. 9). A focal area of demyelination by means of DC compression, as observed in an extramedullary space occupying lesion (Harrison and McDonald, 1977), could account for the loss of preoperative tibial nerve SEPs in this patient. Postoperative worsening of sensory or motor performances and SEPs was not uncommon in this study since it was observed in six out of 20 patients. Surgical manoeuvres to achieve a complete removal of the tumour may account for postoperative SEP deterioration considering that four of these six patients developed a segmental loss of pain and temperature sensation in the upper limbs postoperatively. However, only SEP monitoring during surgery could give further insight to the mechanisms of SEP deterioration.

Mechanism of N13 reduction

The mechanisms which can account for the abnormality of DH responses reflected by reduction or absence of the N13 potential are: (i) infiltration by the tumour itself; (ii) compression by the tumour or cyst; (iii) displacement of the cord causing a rotation of the N13 dipolar field. Only the latter mechanism can be reasonably discarded on the basis of the argument that SEP recordings with a circular array of cervical electrodes did not show, in patients with no identifiable N13 on the conventional Cv6-AC montage, any evidence of a change in the orientation of an active N13 dipolar source (*see* Fig. 3). The progressive recovery of N13 observed after surgery in four patients suggests that the loss of N13 does not necessarily reflect irreversible damage to the DH circuitry; cyst deflation is likely to explain this recovery in two of them (Cases 8, 15) whose tumours were located below the Cv7 level, and topped by a cervical cyst. This explanation does not hold, however, for the two other patients whose tumoral mass was either clearly invading the cervical cord below Cv4 (Fig. 8) or not associated with any cyst (Fig. 9).

Recording strategy of SEPs in intramedullary tumours

The high sensitivity of N13 in intramedullary lesions represents a strong argument to record cervical potentials in all cases where a lesion of the cervical cord grey matter is suspected. In previous publications most authors have recorded cervical potentials using a cephalic reference, either Fz or ear lobe. However, it is now widely accepted that a cephalic reference records the P14 potential which is injected as an 'N14' negativity in the Cv6-cephalic waveform, and thus is inadequate to record separately the spinal N13 and the brainstem P14 potentials. From this work, and others previously published in patients with spinal cord lesions (Mauguière and Ibáñez, 1985; Emerson and Pedley, 1986; Mauguière and Restuccia, 1991; Restuccia and Mauguière, 1991), our belief is that the use of a Cv6-AC derivation represents the best and most reliable means to overcome this problem. This derivation enhances the amplitude of the N13 potential which is not contaminated by the P14 potential, thus permitting an accurate assessment of cord dorsal horn dysfunction.

Combined recordings of median and tibial nerve SEPs permit separate assessment of the conduction in DC fibres and the segmental responses of DH neurons at the cervical level, thus providing the clinician with functional information, which is not given by neuroimaging techniques and can be missed by clinical examination. The 85% rate of abnormal responses justifies the use of SEPs as a screening test in any patient in whom this pathology is suspected.

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BOOK REVIEWS

Guillain-Barré Syndrome. By Richard A. C. Hughes. 1990 Pp. 308. Heidelberg: Springer Verlag. Price £75.00

The Guillain-Barré syndrome (GBS) is an acute monophasic paralytic syndrome affecting the peripheral nervous system. It has challenged clinicians because it is frequently misdiagnosed in the early stages of the illness and may be fatal unless skilfully managed. It has also intrigued research workers because it has the hallmarks of an autoimmune disorder of the peripheral nervous system although the specific antigen and the immunological mechanisms involved are tantalizingly elusive.

The syndrome was clearly described by Guillain, Barré and Strohl in 1916 when they described the clinical features and characteristic cerebrospinal fluid findings in two French soldiers. The same condition had been described earlier by Landry in 1859 and by others, in the era before Quincke had introduced lumbar puncture. It has been referred to in a number of publications as the Landry-Guillain-Barré-Strohl syndrome but the reasons why the names of the unfortunate Landry and Strohl are now omitted from the eponymous title remain obscure. There was a rekindling of interest in the diseases of the peripheral nervous system in the 1950s with the evolution of new electrophysiological techniques that were important in the diagnosis and understanding of the pathophysiology of peripheral nerve diseases and with the use of nerve biopsy for diagnosis and immunopathological studies. Further stimulus for clinical and laboratory research in GBS has been derived from its apparent association with influenza vaccination in the USA and the success of plasmapheresis and intravenous immunoglobulin in its treatment. A number of variants of GBS have been recognized including Miller Fisher syndrome and chronic inflammatory demyelinating polyneuropathy (CIDP).

In view of the interest in the condition for more than a century, and the intensification of research into diseases of the peripheral nervous system over the past 30 yrs, it is surprising that Professor Richard Hughes' book is the first comprehensive monograph to be published on the subject; it is less surprising that this scholarly work will be difficult to better since the author has extensive experience in the clinical management of the condition and a formidable reputation for his research on the immunological aspects of neurological disease. The book is not confined in its scope to GBS but encompasses the other inflammatory and demyelinating neuropathies as well as experimental work on the immunopathology and pathophysiology of demyelination.

The book opens with an excellent historical account of the syndrome in which due acknowledgement is made to the astute clinical observations of Landry. The inflammatory changes in the peripheral nervous system were first recognized by Leyden in 1880 and later confirmed by the landmark studies of Asbury *et al.* in 1969; they had been overlooked in the important paper in 1949 of Kernohan and Haymaker who had emphasized the presence of oedema in the nerves. Hughes provides clear operational definitions of GBS and CIDP based on his own experience and that of others and he confronts the difficult issue of how to categorize patients whose initial symptoms continue to progress in that grey area from 4 to 12 wks.

As may be anticipated, the chapters on the immunobiology and immunology of the peripheral nervous system are excellent and the author brings both objectivity and extensive experience to his review of the immunological aspects of demyelination in which he separates experimentally proven facts from hypothesis. The section on myelin chemistry was less easy to follow than others but this may reflect the reviewer's lack of basic knowledge of the subject-matter It is disappointing that in spite of the intensive immunological studies that have been done on man and experimental animals with inflammatory neuropathy the pathogenic antigen(s) remains undetected and the respective roles of cell-mediated and humoral mechanisms in the disease remain unclear, in contrast to the neater situation that prevails in myasthenia gravis. The author refers to the messy literature on the subject which he has reviewed with admirable clarity. Experimental allergic neuritis has been intensively studied in many laboratories because of the close similarity between the acute and chronic relapsing types and GBS and CIDP, respectively. The author has made important contributions himself that have identified the specific neuritogenic antigens.

The chapter on pathology deals thoroughly and even-handedly with the fundamental aspects of the condition in which there is macrophage-associated demyelination in a multifocal distribution. The clear photomicrographs illustrating relevant features are derived from the author's own observations. He makes the interesting suggestion that the patchy lymphocytic infiltrations imply a cell-mediated immunopathogenesis, but in those cases where they are sparse or absent antibody-mediated demyelination is a more likely mechanism. Cases with overwhelming predominance of axonal degeneration are considered here and elsewhere in the book and add weight to the contention that GBS and its related disorders are a heterogeneous group which leads to difficulty in pinpointing the fundamental immunological mechanism.

Epidemiological studies of GBS have demonstrated a remarkably uniform annual incidence of the condition in most parts of the world where they have been undertaken, in contrast to those of multiple sclerosis Intuitively one would imagine that the two diseases, one affecting myelin of the central nervous system the other that of the peripheral nervous system, would have a similar immunopathogenesis and aetiology However, they differ not only in epidemiological but also in genetic characteristics since there is no established HLA association in GBS. The association of GBS with vaccination, swine influenza, typhoid, tetanus, rabies and polio is carefully reviewed and practical advice given to the clinician. The role and types of associated antecedent infections are considered, an area of research in which the author has undertaken seminal studies.

The excellent account of the clinical features of GBS and the Miller Fisher variant, including autonomic and other complications, and a comprehensive discussion of the differential diagnosis will be of particular value to practising clinicians. It may be noted in passing that tick bite paralysis occurs in countries other than the USA. The mortality rate of 13% in the UK and France seems surprisingly high compared with that in some other countries. The neurophysiology is well reviewed but in less detail than some other aspects of the disease. The author tackles the difficult matter of why the motor fibres are affected to a greater extent than sensory fibres.

The chapter on the treatment of GBS is an excellent account of the modern management of the condition and is a valuable practical guide for any physician involved in the treatment of patients with the disease. Important points on the management of respiratory paralysis, cardiac arrhythmias and the prevention and treatment of deep venous thrombosis are discussed. Pain is a frequently overlooked symptom of paralysed patients who are unable to communicate their discomfort to attending medical and nursing staff, and useful advice is given about its management. The use of steroids in the treatment of GBS has been controversial and the book was published before the negative results of the author's thorough and extensive multicentre trial of intravenous methylprednisolone were available. A welcome feature of the book is that Professor Hughes does not gloss over difficult matters such as whether a patient with GBS who is not improving may, in fact, have a subacute or chronic inflammatory neuropathy requiring steroid therapy. Plasmapheresis is of proven benefit and reports of the use of intravenous human immunoglobulin have been and will continue to be published. Neurologists tend to lose interest after their patients are discharged from hospital and therefore the section on rehabilitation is a timely reminder that we have a continuing responsibility for the care of our patients after the acute phase of their illness.

Chronic inflammatory demyelinating polyneuropathy is less dramatic in its onset and therefore less well recognized than GBS. Regrettably it is often overlooked or misdiagnosed since it is a treatable condition. The author has proposed his own set of helpful diagnostic criteria for CIDP that have been modified from the previous attempts of others. However, it does still remain a difficult matter in individual cases to distinguish some cases of GBS with a relapse from patients with CIDP who have an acute onset. Professor Hughes does not include cases in his definition with an essentially pure sensory polyradiculoneuropathy although there are undoubtedly such cases who otherwise satisfy the diagnostic criteria of CIDP; it is difficult to fit them into any other diagnostic category

The final chapter deals with other forms of inflammatory neuropathy including HIV infection, vasculitic neuropathy, sarcoid, dysproteinaemias and leprosy. Lyme disease and Chagas' disease could perhaps be included in this list. The addition of an account of the GBS written for patients' relatives and friends prepared by the Guillain-Barré Support Group is welcome.

This is an outstanding book. It covers all aspects of GBS and other inflammatory neuropathies and is particularly valuable because it has been written by a single author with extensive clinical and research experience. The literature is thoroughly reviewed and there is an extensive list of references at the end of each chapter. Professor Hughes has a scholarly, thoughtful and objective approach to the subject and writes in a clear and precise style; he maintains the reader's interest thoroughout by expressing his own personal views, which command respect, on a number of controversial issues. The photomicrographs and line-drawings are excellent. The book is highly recommended to all clinical neurologists, physicians and others involved in the management of Guillain-Barré syndrome and research workers in the field. It is essential reading for those who have a particular interest in diseases of the peripheral nervous system.

J. G. McLEOD

Radiation Injury to the Nervous System. Edited by P. H. Gutin, S. A. Leibel and G. E. Sheline. 1991 Pp. 482 New York: Raven Press. Price $171.00.

Take a forum of neurologists, neurosurgeons and radiotherapists discussing the young patient presenting with fit(s), no certain neurological signs and a biopsy proven low grade glioma in an eloquent brain area and there will be no consensus on radiotherapy. Neurologists, fearing late radiation damage or, at least, late neuropsychological sequelae and sceptical of anti-tumour efficacy will elect no treatment; radiotherapists, clinging to less-than-fat San Franciscan data, will irradiate all cases. The fate of the patient depends on the bias of the consultant whose name is at the end of the bed. So it has been for a decade and I see no hint of a change. The indications for radiotherapy for many intracranial tumours are not universally agreed, and the central nervous system (CNS) morbidity of radiotherapy is feared and cited as a major reason for non-referral. Neurologists should realize that radiation therapy is the most important non-surgical therapeutic modality in the treatment of their 'bread and butter' brain tumours; that it can be safely harnessed; that there are exciting new ways of improving its therapeutic ratio [e.g. focal stereotactic methods, partial transmission block (PTB) technique]; that chemoradiotherapy protocols are interesting in several if not many tumours and finally that neuro-oncology is lagging behind virtually every other clinical subspecialty (including urology and gynaecology!) in the development of well conceived clinical studies. It is bizarre that neurologists can master MRI so quickly and yet bother little to understand radiation biology and therapy (certain MRC studies and paediatric protocols excepted). Radiotherapists should not escape blame either: non-critical acceptance of patients with appalling prognosis for therapy and the occurrence of radiation damage to the CNS by too expeditious prescriptions or less than careful planning bring morbidity that their neurological colleagues do not forget.

I have painted a picture of a country full of nihilistic neurologists and uninterested or cavalier radiotherapists, and called for change. There can be no better way to start than to carry this book by Gutin *et al.* around in one's briefcase for 6 mths. Its cost ($171.00) and weight (482 pages) will remind the bearer to dip into it occasionally and this is the best way. The opening chapters comprehensively review the molecular targets of radiation therapy and the early and late effects of such therapy on normal CNS tissues. It is heavy-going for the novice but if you can ski this porridge, light powder snow lies ahead.

The second section is a comprehensive series of reviews on experimental studies of ionizing radiation on normal CNS tissues. If I have any criticisms they relate to the experimental doses which bear little resemblance to most clinically delivered prescriptions; this is not quite fair, however, as focal stereotactic radiotherapy prescriptions are of the same order and the morbidity encountered in such 'radiosurgery' is due to this type of morbidity. The inclusion of sections on neutrons, charged particle radiation (remember the excellent Boston proton data on clivus chordoma control) and implanted isotopes are all of interest and point to future 'gold standard' therapies in neuro-oncology.

Section three is the most interesting one for clinicians. With an understanding of the groundwork from the first two sections, the chapters on the tolerance of the brain and spinal cord to conventional radiotherapy and factors affecting this threshold must fascinate any reader interested in neurology, oncology or human life. Specific chapters on the tolerance of the visual apparatus, the hypothalamus and on late changes in intellect after cranial radiation all maintain this interest. I teach that the optic chiasm is the body's most 'litigious' region for the radiotherapist and I was pleased to find this subject and the tolerance of the hypothalamo-pituitary axis reviewed with adequate references. The late neuropsychometric data on childhood acute lymphoblastic leukaemia survivors and survivors of oat cell bronchial cancer (of which both groups receive combination cytotoxic chemotherapy and prophylactic whole cranial radiotherapy) caution oncologists devising new protocols involving both modalities; the chapter on drug/radiation neurotoxicity is therefore a welcome addition to this edition of the book. The subject of late effects of radiation on intellect has been one of intense study particularly in relation to late survivors of acute lymphoblastic leukaemia and neuropsychologists have teased out those particular facets at special risk and correlated them, as well as they are able, with radiation dose, fractionation, age at treatment, late CT/MRI changes, etc. These studies have laid the foundation for current generation acute lymphoblastic leukaemia CNS prophylaxis work where we attempt to reduce this CNS toxicity by reducing radiation dosage in lower risk children and attempt to give intrathecal methotrexate before rather than during or after the radiation. Already, improved neuropsychological development has been reported.

The last section disappointed me in that the editors lost the opportunity to review the current different approaches to reduce CNS morbidity in radiotherapy Apart from a chapter on radioprotection, which currently does not show great promise, the subject of practical, clinical techniques for reducing CNS morbidity could have been much improved by a final chapter or two synthesizing conclusions from the previous data. For example, our own work on medulloblastoma patients, whose craniospinal radiotherapy at a young age has led to marked neuropsychological and growth retardation, has led us to introduce a partial transmission block technique. In this technique the whole cranium and spine receives much lower doses per fraction than in conventional craniospinal radiotherapy, whilst the posterior fossa receives the conventional fraction sizes each day. This technique has been devised from appreciation of the data (much of it reviewed in this book) proving that daily fraction size is a vital factor in late neuropsychological sequelae. Therefore, for the propylactic whole cerebrum radiation portals it seemed sensible to attempt to reduce the daily fraction size if it could be done (i) without prolonging overall treatment time, (ii) without altering the fractionating schedule to the posterior fossa and (iii) without jeopardizing tumour cure rates. We have argued elsewhere that the technique achieves these objectives and the neuropsychological follow-up of these children is under continuing review. This is just one instance of our use of the radiobiological data, as reviewed in this book, to adapt a clinical method. A second example is the race to develop fractionated focal stereotactic radiotherapy to avoid the possible extra morbidity of single large fractions. It would have been of great interest to have an overview of such methods.

Notwithstanding these reservations, I consider this book an outstanding collation of the present state of knowledge of radiation tolerance of the human nervous system and I commend it to neurologists and radiotherapists as important reading.

P. N. PLOWMAN

Psychopathology and the Brain. Edited by Bernard J. Carroll and James E. Barrett. 1991. Pp. 315. New York: Raven Press. Price $156.50.

When more than 350 yrs ago, William Harvey first considered the intricacies of the movement of the heart and circulation of the blood, he confided that he found 'the matter so truly hard and beset with difficulties that I all but thought that . . . [they] . . . had been understood by God alone.'

In a field such as the one under review here, it is difficult not to echo Harvey's sentiments The field demands optimism and courage, qualities which are not lacking among the authors of this volume. The volume consists of contributions to a meeting of the American Psychopathological Association in 1989. The chief advances in neuroscience over the past decades have arguably been technological rather than conceptual; 'the gain in brain', it has been said, 'lies mainly in the stain'. In view of this, a book such as this which describes the state of play in 1989 is likely, 2 yrs later, to have been overtaken by events. The question must be whether there is sufficient conceptual novelty to justify its place among an already overcrowded reading list.

We are presented here with selective coverage of five main areas of current interest, each of approximately equal length and each containing contributions from two or three groups of workers. There are no contributions which deal exclusively with genetics, molecular biology or dementia. All the contributions are from North America. This is reflected in the citations, but the European contribution is fairly acknowledged.

The opening section contains two contributions to the discussion of 'frontal-limbic striatal mechanisms in psychosis', where the term psychosis is generally taken to be synonymous with schizophrenia. Patricia Goldman-Rakic, who is well known for her tracer studies on the topography of the frontal lobes of monkeys, applies some of her ideas and findings to memory in schizophrenia. She relies heavily on the concept of working memory. This model, which replaces the concept of short-term memory, envisages a three-part construct with a 'master' central executive which controls two 'slave' systems, one subsuming visuospatial elements, the other phonological loops. Goldman-Rakic's thesis is that schizophrenia and damage to the prefrontal cortex have psychological mechanisms in common, expressed in various ways but all predicated to some degree upon deficits in working memory.

When the future James II viewed Wren's new St Paul's Cathedral, he is said to have called it 'awful, amusing and artificial'. He was being as complimentary as he could: the building instilled in him a sense of awe and amazement; the skill of its conception and execution rivalled Nature by its artifice Clearly, these meanings have shifted, but in calling Goldman-Rakic's contribution, together with much of that described in the rest of the book, artificial, I hope to retain something of the old meaning without altogether dispensing with the current one. A model as artificial as those with which we are presented here, has the ability to attract funding for impeccably contrived experiments in answer to readily definable questions. The very serious question is whether such experiments in fact lead to entirely trivial answers. A second problem, the seriousness of which becomes more vivid when considering the research presented in this book, relates fundamentally to psychiatric nosology itself. In some of the contributions in this book, this problem is confronted and changes in psychiatric nosology are proposed In others, and this contribution of Goldman-Rakic seems to be one of them, a term such as 'schizophrenia' is used as if it were a disease rather than a concept. Whatever the criteria, problems of definition bedevil the field and, unless one is researching specific or individual symptoms, can lead to some confusion of mind. In the next contribution of this section, Daniel Weinberger discusses intercortical connectivity in the construction of a model which may prove to have considerable relevance to the studies of the psychoses, in particular schizophrenia. He has made his chief conceptual point before, but it bears repetition. He draws attention to the observation that psychiatric illnesses have a staggered age of mean onset For example, a mean age of onset of schizophrenia occurs in the early to mid-twenties, whereas affective psychoses have a mean onset 10 to 12 yrs later, and Weinberger's suggestion that one should pay attention to neuro-developmental and neuro-maturational factors is clearly important. There is more here than a simple technological advance. The placement of cerebral development in the conceptual limelight promises new insights for the future.

The next section in the book deals with the psychopathology of disorders of the basal ganglia. Stephen Wise opens with a lucid exposition of the basic anatomy and physiology of these curious structures. It is well-referenced, albeit to 1988, and contains a brief coda introducing the possibility of a role for the basal ganglia in cognition. The treatment is not intended to contain original insights, but prepares the ground most effectively for the account of Huntington's disease by Susan Folstein and her co-workers which follows.

The neuropathology and genetics of this condition have received considerable attention in recent years, and the quantitative nature of the fall-out of small neurons in the caudate and putamen has been clarified to the extent that pathological grading of the disease is now possible. Folstein *et al.* discuss the relationship between Huntington's disease and affective disorder. Affective disorder is often the earliest harbinger of Huntington's disease, and is indistinguishable phenomenologically from uncomplicated depression. Other psychoses, in particular paranoid psychoses, also occur but affective disorder, both mania and depression, is considerably more common and is not simply the reflection of an adjustment problem to a terminal illness. Folstein attempts to integrate her ideas about the affective disorder with the dementia and motor disturbance which complete the diagnostic triad of the condition. Her observations are based on neuro-imaging and neuropsychological tests and not on clinico-pathological correlations. A linkage of the two approaches is awaited.

Judith Rappoport concludes the section on the basal ganglia with a brief review of some of the evidence linking obsessive compulsive disorder with basal ganglia dysfunction. There are now active research programmes in many centres directed at obsessive-compulsive phenomena and abnormalities in the functional imaging of the basal ganglia, especially the caudate nucleus in some of the tic disorders such as Gilles de la Tourette Syndrome, have been found. Such findings result from the pooling of clinical and imaging studies which have outstripped any structural, neuropathological findings. Rappoport proposes a model whereby the normal state of recognition of stimuli by the basal ganglia is coupled with an innate releasing mechanism which triggers what she terms a 'species-typical' behavioural response. This sequence is effectively uncoupled in these disorders. The model may be heuristically valuable, but there is something not entirely convincing about it. It appears to explain or incorporate all current observations but in fact predicts very little. Of course it would be quite unfair to single out Rappoport in this regard; the same is true of many, if not most, of the models represented in the remainder of the book. In Rappoport's case, there is a further complication, which she admits, that obsessive-compulsive phenomena are both more variable and aetiologically diverse in ways that are not immediately associated with basal ganglia disorder.

The central section of the book consists of two parts, each containing three contributions. The accent in this section is on behavioural studies. It cannot be emphasized enough that these and similar studies are based on animal work, particularly involving the ubiquitous laboratory rat, and only a few cases are underpinned by work on humans. This is a fact of life but in issues as complex as psychopathology even more care must be taken in making interspecific extrapolations and in particular equating or paralleling human emotional states with those presumed to exist in the animal subject. None of the contributions in this section escapes criticism. The first, which deals with behavioural biology and development, contains presentations on the role of stimulation in infancy, 'hidden' behavioural regulators in development and long-term neural consequences of stress during development. All three contributions emphasize that mother-infant interactions are important for the future well-being of the individual. Kuhn and Schanberg examine the role of touch in development and emphasize its importance. Their work is derived from investigation of the developmental consequences of handling rat pups. The results are inevitably difficult to interpret, but the emphasis that touch is important for adequate development is rightly emphasized. Next, Myron Hofer explains that 'hidden' behavioural regulators may be acting much earlier in development, in what he terms a 'prelearning phase', implying that in the earliest post-natal period (maybe even before) the stage is set for future and perhaps abnormal development. The notion is carried further by Thomas Insel who postulates a kind of early chemical or hormonal imprinting in which stressors in early life are able to modify the developing neurohumoral system of the brain. He posits a critical period of development particularly vulnerable to disturbances which may affect neuronal migration and/or proliferation resulting in abnormalities which are displayed only in later life. Again this work is based on observations in rats, but Insel points out that critical phases in early development are likely to occur in humans. Drugs taken by the mother could result in such imprinting. Insel conceives that the median eminance-pituitary-adrenal axis may be particularly vulnerable in this regard, setting up potential vulnerability to depression in later life.

Some years ago, a newspaper competition invited contestants to supply openings to newspaper editorials designed to discourage one from reading on. If there were a similar competition for scientific titles, I should have to give honorable mention to Robert Adamec's 'anxiety personality in the cat . . .', which opens the next part of the middle section, dealing with personality and 'biobehavioural systems'. Essentially Adamec suggests that a 'possibly' genetically based commonality of behaviours related to defence against threat allows one to examine any of the relevant species (in his case, the cat) in order to provide meaningful extrapolations to the others. King, Clark and Mefford in their offering 'neurochemical studies of impulsive-aggressive personality traits', take the matter further.

Their basic postulate is that 'impulsive' personality traits in man are caused by increased levels of dopamine activity. There is a problem with definition here and King and his collaborators describe 'impulsive' behaviour as a portmanteau containing elements of histrionic, borderline and narcissistic personality disorders. These are American concepts and, although they are daily employed in British psychiatry, there are purists who do not feel that they offer any advantages over the European classifications. The term 'impulsive' probably equates partially to the term 'explosive' as used in European work. The other problem is that case definition of the personality disorders is far less reliable even than that of the major psychoses as alluded to above. In King *et al.*'s work 'impulsive' human subjects are subjected to repeated lumbar puncture in order to assess CSF dopamine, a procedure which depends on the often fragile goodwill of the subjects! They also use a non-invasive method of guessing at mesolimbic dopamine activity by observing locomotor activity.

Their findings, while not easy to interpret, show that 'CSF dopamine levels are positively correlated with extroverted traits' and that average daily motor activity is positively correlated with 'histrionic traits'.

Both Adamec and the King group are influenced by the ideas of Robert Cloninger, whose chapter on brain networks underlying personality development completes the middle section. The prolific Cloninger has developed a simplified typology of personality which he asserts is more in keeping with what is known about the genetics of personality than some of the other dimensional theories of personality such as that of Hans Eysenck. This chapter provides a useful introduction to Cloninger's thoughts on personality, although the bulk of it has appeared elsewhere. The model postulates the existence of three main personality dimensions: harm avoidance, novelty-seeking and reward dependence. Typical characteristics of each dimension are described; for example, a 'high-scorer' on harm avoidance would be shy, cautious, inhibited and so on, a 'low-scorer' on the same dimension would be confident, carefree, uninhibited, etc. Such dimensions correlate well with emotional types, according to Cloninger, and are reproducible across sexual and racial boundaries. The next step is to find three 'major brain systems' to correspond to these dimensions. Cloninger believes that he has done so. Novelty-seeking is a function of the 'behavioural activating system', harm avoidance of the 'behavioural inhibition system', and reward dependence is a function of the 'behaviour maintenance system'. Cloninger presents these symptoms as subgroups of the 'limbic system', and draws their putative connections in diagrams of considerable complexity. He accepts that the systems overlap, but asserts that each, at bottom, has a neurotransmitter as its chief modulator. Behaviour activation relies chiefly on dopamine, inhibition on 5-hydroxytryptamine and maintenance (although this does not appear explicitly) on noradrenalin. Cloninger does not, it must be admitted, claim that matters are as simple as that, and he accepts that there are difficulties in relating genotype with phenotype in something as complex as personality The other problem implicit in Cloninger's model is his use of the term 'limbic system'. Although this is in common currency, not all anatomists would accept the existence of the system without reservation. The late Alf Brodal, for example, made a very persuasive case that the individual structures within the limbic system have such a plethora of different functions and connections that to think of them as a system is not meaningful. Nevertheless, Cloninger does make a thoughtful attempt to, as one of the discussants had it, 'relate the hyperspace of personality to brain function . . . and other biological underpinnings'

The final section of the book deals with the neurobiology of affective disorders. It opens with a contribution from Harold Sackeim which is concerned with hemispheric lateralization of mood and emotion. It is well accepted that the hemispheres have differing functions, but, equally clearly, there is also a great deal of overlap, so that the evidence for hemispheric lateralization of mood is often contradictory. The classic studies appear to point to a right hemisphere dysfunction as being of aetiological significance in depression, but there is a body of later work by Cutting, for example, which receives little attention from Sackeim, which points to the left hemisphere as the chief culprit in depression. In the main, Sackeim delivers a balanced and well-referenced guide to this difficult and often contentious subject, and his chapter may be recommended to anyone wanting a mainstream introduction to the field.

The next contribution, from Coffey and Figiel, focuses on the neuropsychiatric significance of subcortical encephalomalacia. This term refers to the hypolucency of subcortical white matter which is often observed on CT scans, especially in the elderly. The finding is reflected in enhanced signals on T_2-weighted MRI scans. In the past, these findings have been assumed to denote Binswanger's disease, a form of hypertensive vascular dementia characterized pathologically by confluent subcortical white matter infarction. Such a universal attribution will no longer suffice, and Coffey and Figiel provide a detailed and thought-provoking review of the subject, making it clear that these radiological findings are found in other settings and may be of wider significance. In an exemplary presentation, they provide the background for informed appreciation of the area, together with suggestions for future research. It is illustrative of some of the best research approaches currently on the neuropsychiatric scene.

The book ends with Bernard Carroll's essay on the psychobiology of manic depressive disorders. Most of the essay consists of a straightforward review of the history and phenomena of the concept of manic depression or affective disorder, together with an attempt to integrate neuropsychological concepts of 'consummatory reward', 'incentive reward', 'central pain' and 'psychomotor regulation'. The influence of Cloninger is acknowledged, but the analysis extends beyond that. Carroll in effect suggests that it is necessary to deconstruct the current concept of affective disorder allowing the resultant reformulation to stimulate research. Although it is still early days, it seems that an approach of this kind is likely to be fruitful, although the disruption to one's received notions of diagnosis is likely to be rather unsettling.

In conclusion, does this book contain enough of lasting value to justify its high purchase price, given that many of the results contained therein have been overtaken by subsequent reports? For the individual purchaser, the answer is probably no. Most of the conceptual work is available in the mainstream literature. Nevertheless as a synthesis of perhaps the less glamorous but no less exciting areas of concern within neuropsychiatry, the book has undoubted value. Individual insights of considerable importance are scattered throughout the contributions. Anyone seeking to enter the field has in this book a valuable, if selective, account of work up to the late 1980s, which can act as a springboard from which to dive into the primeval soup which is the present literature. The book should be available in all libraries of institutions where neuropsychiatry plays an active part. Its appeal to the general reader is less clear.

MICHAEL ISAAC

Delirium: Acute Confusional States. By Zbigniew J. Lipowski. 1990. Pp. 490. New York: Oxford University Press. Price £60.00

It is estimated that some 10–15% of inpatients suffer from delirium at some point of their hospital admission. It is therefore remarkable that, apart from a treatise by Frings, published in 1746, and an earlier edition of this book, no other substantive works on delirium have been produced. Further, in an era of such progress, it is a salutary comment from Lipowski that no major advances in the understanding of delirium have emerged in the last decade.

In this reviewer's opinion, all books of this type should begin with an historical account of the subject, allowing the reader to understand the development of central concepts from the perspective of changing ideas over time. Lipowski does this admirably, having done a considerable amount of searching amongst primary texts of relevance. One of the outstanding facts that emerges, is that delirium, or a syndrome resembling it, has been characterized since time immemorial. How is it then that so little attention has been given to such an apparently important and common condition?

Some answers are found in the historical introduction. Thus, delirium is found mainly in general medical and surgical settings, and has largely escaped the attention of those interested in the brain. It was poorly observed by the 19th-century alienists, who managed, by and large, chronic mental disorders, and, falling as it does at the interface of neurology and psychiatry, it was ignored by both disciplines in this century, with the consequent development of confusion about terminology, presentation and management.

There were some notable exceptions. Willis recognized delirium as a symptom rather than a disease. By the 19th century, clouding of consciousness had become recognized as one of its hallmarks, and Hughlings Jackson used examples of delirium to develop his theories of brain organization and consciousness, the resultant model being applicable to mental disorders more generally. By this time, the earlier synonym, phrenitis, which had reference both to the diaphragm and the brain, had long lost favour, and the cerebral nature of the condition was undoubted.

The most outstanding work this century was that by Romano and Engell, which, according to Lipowski, represented a turning-point in the development of the concept. They were the first to embark on a scientific enquiry of the pathogenesis of the condition.

The term delirium was used in English for the first time in 1592. It remains today Lipowski's preferred term, and has now become enshrined in DSM III, and its successors. It combines states earlier separated on no good grounds, such as those characterized by lethargy and hypoactivity as opposed to excitement; it recognizes the inclusion of delirium tremens within its penumbra, and it does away with the 30 or more inconsistently used and poorly described synonyms found in the medical literature.

Lipowski dislikes the commonly used 'acute confusional state', and deprecates any trend to associate this term with the hypoactive, psychomotor retarded patient, and delirium with the hallucinated and agitated. He offers the following definition of delirium:

'Delirium is a transient organic mental syndrome of acute onset, characterized by global impairment of cognitive functions, a reduced level of consciousness, attentional abnormalities, increased or decreased psychomotor activity and a disordered sleep wake cycle.'

As such, his definition is succinct, and more helpful than the diagnostic criteria laid out by DSM IIIR, which frankly are misleading. The key features are the transient nature of the presentation, the evidence for an organic factor and the global impairment of cognitive functions

Lipowski takes us through the rich permutations of the clinical features, distinguishing three variants: the hyperactive, hyperalert; the hypoactive, hypoalert; and the mixed. The hypoalert picture is often missed, or the patient incorrectly diagnosed as being depressed. It is incidentally often the reports of the nursing staff that are vital in detecting delirium in its early stages, and give-away clues, such as the alteration of sleep pattern, which should always arouse suspicion (so-called sun downing, when the sun goes down the patient gets up).

Although some impairment of consciousness is central to the condition, Lipowski deplores the phrase 'clouding of consciousness', better to speak of levels of conciousness or awareness '. . . to imply how readily he or she responds to stimuli, maintains the waking state, is able to process and store information, and is capable of acting purposefully'.

The rest of the first part of the book covers in some detail the multiple aetiologies, the speculations on pathogenesis (it was good to read the works of Bernard Hart quoted as summing up the position today as: 'Of the precise processes by which delirium is mediated we know nothing;' Hart was the first psychiatrist to have a position at Queen Square, and an early practitioner of what later became liaison psychiatry), diagnosis and its differential, especially from other organic mental syndromes, and treatment (haloperidol is Lipowski's drug of choice)

Part two is a detailed review of many of the organic causes of delirium, while part three covers special patient populations, such as the elderly, the post-operative and those who have been burned or are post-partum.

This excellent book, nicely produced by Oxford University Press, which covers such an important area of clinical practice, should be read by anyone who deals with acute hospital patients, and certainly by neurologists and psychiatrists. There is a lot of interesting information contained within it, and much clinical wisdom. Lipowski brings order to confusion.

MICHAEL TRIMBLE

Neuropsychology and the Dementias. By S. H Hart and J. M. Semple 1990. Pp 343. London: Taylor and Francis Price £29.95.

The aims of this book on the neuropsychology of dementia are ambitious. The first is to highlight some of the major advances in the understanding of dementia and to consider their implications for future research and treatment. A second aim is to show how work from traditionally disparate disciplines is being integrated within dementia research in an unprecedented way and a third is to falsify the long held view that the study of dementia will not advance the knowledge of brain behaviour relationships because of the diffuse nature of the disorder. Although the main thrust of the book is an analysis of the neuropsychological abnormalities accompanying Alzheimer's disease, the authors, in order to address these aims, have also tackled other neuroscience topics in relation to a variety of dementing conditions. Their second and third aim is founded upon the argument that the neuropsychological study of dementia has somehow brought neuropsychology away from being a relatively isolated and inaccessible discipline into being a key player in neuroscience research. This is certainly true.

First, in neuroscience we have relatively few techniques to bring to bear on the understanding of human brain function. These include neuroendocrinological and neuropharmacological probes to investigate neuroreceptor status; electrophysiological paradigms to investigate concerted regional neuronal activity; functional brain imaging to elucidate regional metabolism and thus energetic neuronal processes; and neuropathology to provide direct information about the involvement of individual brain areas in disease. Although findings from these various methodologies are often related to simple descriptions of behaviour or clinical status they are most fruitful when they are performed in conjunction with a detailed neuropsychological analysis of behaviour. An obvious example of this concerns the use of functional brain imaging to examine regional abnormalities underlying various neurological and psychiatric disorders. Findings of regional abnormalities are abundant in the literature but they are often contradictory, inconsistent and fail to provide a clear relationship to clinical symptoms and signs. The recent application of paradigms derived from the field of cognitive psychology to activate certain brain regions during functional imaging in both normal volunteers and patients with neuropsychiatric disorders has allowed a better understanding of the differential involvement of neuronal systems underlying cognitive processes and has allowed inferences to be made about the nature of abnormalities highlighted in various clinical groups. Thus cognitive psychology, a discipline which heretofore has involved the fractionation of human behaviour into component psychological processes without reference to neuroanatomical brain systems, is now providing an increasingly recognized critical methodology for understanding the neuronal basis of normal and abnormal behaviour.

Secondly, when neuropsychology has been applied to the relationship between brain structure and function in a clinical setting, it has tended to involve single cases with relatively well localized brain lesions Because dementia, especially Alzheimer's disease, is now such a common and important problem, psychologists have now turned their attention to this disorder By applying expertise gained from these single case studies, important insights have been provided not only into the nature of the fundamental abnormalities underlying the dementias but also into the application and measurement of therapeutic strategies In turn, clinical scientists have recognized the important contribution of neuropsychology as a discipline; it now has a firm place in the repertoire of neuroscience investigative techniques.

In this book Hart and Semple have amply demonstrated these points. In the first chapter, the major branches of psychology, including cognitive psychology and clinical neuropsychology, are described. There follows three chapters devoted to accounts of the neuropathology, neurochemistry and psychopharmacology of dementia. Although they are brief and do not claim to be comprehensive, they cover the main findings in these areas. In order to be able to elaborate their later discussion which shows how the various dementing conditions have unique profiles of neuropsychological deficits, at least in the early stages, they cover a wide variety of syndromes which include, in addition to Alzheimer's disease, Pick's disease, vascular dementia, Parkinson's disease, progressive supranuclear palsy, Huntington's chorea, Creutzfeld-Jacob disease and alcoholism. A further two chapters are devoted to clinical descriptions and the differential diagnosis of these disorders. Having set the scene, five chapters are then devoted to neuropsychological findings in the realms of memory, language, attention, sensation and perception, and movement and praxis. The first aim of the book is achieved in these chapters. First the literature reviewed is comprehensive and is structured in a meaningful way. For example, at the beginning of each chapter the authors briefly describe the various current concepts relating to each of the cognitive disciplines and propose, with these in mind, a format with which to address the dementia literature. The dementia literature is therefore described and discussed in the context of modern neuropsychological theory. At the end of each chapter is a concluding paragraph which synthesizes the main findings and suggests ways in which these can be applied to the management of individual patients. By indicating deficiencies in present studies they provide pointers towards areas requiring further research. The final chapter is devoted to an appraisal of rehabilitation and management strategies and ranges from an assessment of cognitive methods, such as memory aids, to the prospect of brain transplantation. This book therefore fulfills its aims. A possible problem is that it is too detailed with respect to neuropsychology for an audience of general clinicians and not detailed enough for established experimental neuropsychologists. However, by reading individual chapters of the book both clinicians and scientists should be able to find something new and relevant. The book as a whole is ideally suited for a newly qualified psychologist or psychiatrist who is embarking on a research career into the neuropsychology of the dementias.

EILEEN M. JOYCE

Migraine and Other Headaches: The Vascular Mechanisms. Frontiers in Headache Research. Volume 1. Edited by Jes Olesen. 1991. Pp. 384. New York: Raven Press. $112.50.

If any field in neurology has affected a large patient group and yet proceeded to therapy with little or no scientific basis then this must be the management of headache, particularly migraine. Much has changed to redress this in the last 5 yrs and much of what has been done has been recorded in the volume. The clinical observation that patients complain of throbbing pain has led clinical researchers to examine vascular mechanisms in headache and this is the specific aim of the book. This book arose from a meeting held in Copenhagen in late 1990 to which most of the people who had contributed to the field had been asked to attend and participate. Professor Jes Olesen, the editor and architect of the meeting, was the ideal person to do so being pivotal in the most important studies that finally gave migraine a biological measureable change, i.e. reduced cerebral blood flow during the aura. With typical frankness and scientific honesty Olesen pitted those with opposing views in similar sessions and provided very ample time for each to discuss the weakness of the other's position. The resulting record is a careful and remarkably balanced review of the topic.

The book commences with a presentation and discussion of the methods that have been used to determine cerebral blood flow in headache subjects. Dr Niels Lassen, arguably one of the most influential and formative workers in the field of cerebrovascular physiology reviewed the Xenon method for which he is largely responsible. The whole of the first section of the book deals with the many pitfalls of performing these studies and sets the scene for the arguments to follow. I found the presentation in the section of the book dealing with radiation doses, a refreshing consideration of the studies with the patients' interests placed first. This chapter serves to remind researchers in the field that our subjects are our patients, that they are basically well and must not be subjected to excesses in order to solve their problems, *primum non nocere*. Lassen's discussion and summary on methods sets the tone for these sections throughout the book.

Short and to the point, it summarizes issues raised during the very valuable discussion sessions and has the benefit of Lassen's mature intellectual filter

The rest of the book deals with vascular mechanisms divided up on a logical clinical basis to address the various facets of migraine. Throughout the meeting, and now in the book, Olesen was careful that migraine with aura (*classical migraine*) and migraine without aura (*common migraine*) should not be mixed when results or interpretations were made. The chapters reflect this organization and make checking small points in either topic easy Thus the book deals with vascular changes in interictal studies of migraine with aura, then with the aura phase and then with changes seen during spontaneous headache. Similar sections are to be found concerning migraine without aura. Additional sections dealt with the mechanisms of migraine with aura, transcranial Doppler studies and a separate section was devoted to Cluster Headache. A last important section addressed the interaction of the anti-migraine drugs on cerebrovascular mechanisms. Some of the specific chapters and topics deserve more detailed mention.

The oligaemia that was first characterized by Olesen *et al.*, and remains one of the few scientifically *hard* biological features of migraine, was discussed for a whole session. The point is made by a number of the authors that the focal oligaemia is usually seen in parieto-occipital cortex in what seems to be a middle cerebral/posterior cerebral artery watershed region. Dr Friberg and Dr T. S. Olsen expounded completeley contrasting views on whether the aura phase was due to ischaemia or was oligaemic without reaching ischaemic levels. The question of error of measurement due to Compton scatter was discussed at the meeting perhaps to excess but has been represented in a very balanced way in the book. In brief, the argument is that the Xenon method overestimates regional cerebral blood flow because detectors receive input from areas of high and low flow and the averaging produced leads to an overestimation of flow. Thus Olsen argues that the area of focal oligaemia is indeed ischaemic and that ischaemia has a primary role in the pathogenesis of migraine. While this reviewer does not find that view compelling, one of the strengths of this book is the very balanced preparation that has opposing views argued equally by those actually doing the work

Basic neuroscience studies were included in the section on mechanisms and particularly addressed the mechanism of migraine with aura. Again the most prominent workers review such issue as spreading depression (Dr Martin Lauritzen) and the role of the trigeminal primary sensory afferents (Dr Michael Moskowitz). The very interesting and highly promising technique of magnetoencephalography was introduced by Dr Greg Barkley and Professor Michael Welch. This technique may enable *in vivo* measurements that seem to behave like the phenomenon of spreading depression. The convincing demonstration of spreading depression in man would settle a fundamental question in migraine research. Some clinical studies should be mentioned under the broad rubrick of pathogenesis. The meeting (and the book) were particularly fortunate to have Dr Rune Aaslid speaking on transcranial Doppler. He is one of the pioneers in the field and explained very complex concepts with the ease that those who understand a matter very often do. Studies using transcranial Doppler in migraine are presented and Dr Nyberg-Hansen gives an excellent summary.

This book is a balanced clinical and scientific account of a complex and quickly changing field. Most of what is included is not in textbooks but much of it will be in the coming years. It would thus make an excellent addition to any library until those textbooks are written. For clinicians interested in a subject that is receiving much attention in the popular press this book will fill all the gaps from medical school.

PETER J GOADSBY

ANNOUNCEMENTS

Courses

The following courses are being organized by the Institute of Psychiatry (University of London), the Maudsley Hospital and Bethlem Royal Hospital:

'Sixth Annual two-day course on eating disorders', September 21–22, 1992.

Women and Mental Health—Women and Community Care—a Multidisciplinary Approach', November 26–27, 1992

For further information contact Mrs Lee Wilding, Conference Office, Institute of Psychiatry, De Crespigny Park, London SE5 8AF (Tel 071 703 5411, ext. 3170, Fax. 071 703 5896).

The Medical College of Wisconsin and Continuing Medical Education, Inc announce the seventh in a series of courses 'The Sharper Image in MRI' MR Angiography and Imaging of the Head, Spine and Musculoskeletal System, July 27–31, 1992, at the Broadmoor Resort in Colorado Springs, Colorado, USA Fees $495.00 before June 5, 1992, $545.00 after June 5, 1992. Contact. Marti Carter, CME, Inc , 11011 W North Avenue, Milwaukee, WI 53226, USA (Tel 414 771-9520)

Conferences organized by The New York Academy of Sciences

'The Neurohypophysis: a Window on Brain Function' at Dartmouth College, Hanover, New Hampshire, USA, July 16–20, 1992

'The Melanotropic Peptides' at Palais des Congrès, Place de la Cathédrale, Rouen, France, September 6–9, 1992.

'Symposium on Temporal Information Processing in the Nervous System with Special Reference to Dyslexia and Dysphasia' at Vista Hotel, New York City, USA, September 13–16, 1992.

'The Role of Insulin-like Growth Factors in the Nervous System' at Crystal Gateway Marriott, Arlington, Virginia, USA, November 4–7, 1992.

'Molecular Basis of Ion Channels and Receptors involved in Nerve Excitation, Synaptic Transmission and Muscle Contraction' at the Ibuka Memorial Hall, Waseda University, Tokyo, Japan, January 12–15, 1993.

'Slow Infections of the Central Nervous System: The Legacy of Dr Bjorn Sigurdsson' at Háskólabió Conference and Cultural Centre, Reykjavik, Iceland, June 2–5, 1993.

For further information contact the Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA (Tel: 212 838-0230; Fax 212 888-2894)

Ultrasound Angiography 92—2nd International Conference

This conference will be held in the Medical and Biological Sciences Building, University of Southampton, UK, September 2–4, 1992. For further information contact Conference Secretariat, Ultrasound Angiography 92, PO Box 15, Eastleigh, Hampshire SO5 5XG, UK

The First International Symposium on Brain Death

This symposium will be held in Havana, Cuba, September 22–25, 1992 For further information contact 1st International Symposium on Brain Death, Palacio de las Convenciones, Apartado 16046, La Habana, Cuba (Telex 511609 palco cu, Fax: 22-2382

The Second International Congress on Objective Assessment in Rehabilitation Medicine—Low Back Pain and Brain Injury

This congress will be held in Montréal, Québec, Canada, October 5–6, 1992. For further information contact Centre de formation en réadaptation du Québec, 6300 Avenue Darlington, Montréal, Québec, Canada H3S 2J4 (Tel: 514 340-2089, Fax 514 340-2149).

International Congress on Neurology and Neurosurgery at the Third International Symposium on Epilepsy

These two events will be held in Havana, Cuba, December 7–11, 1992 For further information contact Professor Otto Hernández Cossio, President of the Organizing Committee, Apartado 4248, Zona Postal 4, Ciudad de la Habana 10400 Cuba (Tel: 32-7825, Telex: 511609 palco cu; Fax: 22-8382 and 20-2350).

AAEM 1992 Annual Meeting

The Thirty-Ninth Annual Scientific Meeting and the Fifteenth Annual CME Courses of the American Association of Electrodiagnostic Medicine (AAEM) will be held in Charleston, South Carolina, USA, October 14–18, 1992 For information contact The AAEM Office, 21 Second Street SW, Suite 306, Rochester, MN 55902, USA (Tel 507 288-0100, Fax 507 288-1225).

MRI Update 1993: MR Imaging of the Head, Spine and Musculoskeletal System

The eighth in a series, this continuing education course is being presented by the Medical College of Wisconsin and Continuing Medical Education, Inc at the La Quinta Golf and Tennis Resort in La Quinta, California, USA, February 22–26, 1993 For information contact Marti Carter, CME, Inc., 11011 W North Ave, Milwaukee, WI 53226, USA (Tel: 414 771-9520).

The Eighth Conference of the South East European Society for Neurology and Psychiatry

This conference will take place in Jerusalem, Israel, February 28–March 3, 1993. For information contact Professor S Feldman, The VIII Conference of the South East European Society for Neurology and Psychiatry, c/o PO Box 50006, Tel Aviv 61500, Israel (Tel: 972 3 5174571, Fax: 972 3 655674).

The Fourth International Bethel-Cleveland-Epilepsy Symposium: Seizures and Syndromes

This symposium will be held in Bielefeld, Germany, March 11–14, 1993. For information contact Dr S. Noachtar, Epilepsie-Zentrum Bethel, EEG Abteilung, Maraweg 21, 4800 Bielefeld 13, Germany (Tel 05 21/1 44-36 99).

The Seventh Annual Magnetic Resonance Imaging Conference

This conference, organized by the Barrow Neurological Institute of St Joseph's Hospital and Medical Center, will be held at the Arizona Biltmore Resort Hotel, Phoenix, Arizona, USA, March 14–17, 1993. For information contact Maria Sodaro, R.N , B S N., Research and Education Coordinator, Radiologic Education Center, St Joseph's Hospital and Medical Center, 350 W. Thomas Road, Phoenix, AZ 85013, USA (Tel: 602 285-3956).

The Second International Conference on Stroke

This conference will take place in Geneva, Switzerland, May 12–15, 1993 For information contact N. M Bornstein, MD, International Conference on Stroke, c/o Kuowi Travel Ltd, 7 Rue de Berne, CH-1211, Geneva 1, Switzerland (Tel. 41 22 732 0888; Fax: 41 22 731 5078).

The Ninth International Symposium of Brain Edema

This symposium will be held in Tokyo (Pacifico Yokohama), Japan, May 16–19, 1993. For information contact Umeo Ito, MD, Department of Neurosurgery, Musashino Red-Cross Hospital, 1-26-1, Kyonan-cho, Musashino-shi, Tokyo 18, Japan (Tel: 81-422-32 3111, Fax: 81-422-32 3525).

The Seventh World Congress on Pain

This congress, sponsored by the International Association for the Study of Pain, will be held in Paris, France, August 22–27, 1993 For information contact Louisa E Jones, International Association for the Study of Pain, 900, NE 43rd Street, Suite 306, Seattle, WA 98105, USA (Tel: 206-547-6409; Fax 206-547-1703)

The Thirteenth International Congress of EEG and Clinical Neurophysiology

This congress, organized by The International Federation of Clinical Neurophysiology, will be held in Vancouver, BC, Canada, August 29–September 4, 1993. For further information contact Information Secretariat, 645-375 Water Street, Vancouver, BC, Canada, V6B 5C6 (Tel: 604-681-5226; Fax 604-681-2503)

The Second World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) Meeting

This meeting will be held in Los Angeles, California, USA, September 8–11, 1993. For information contact Dr Om P Sharma, Room 11-900, LAC/USC Medical Center, 1200 N. State Street, Los Angeles, CA 90033, USA (Tel: 01-213-226-7923; Fax: 01-212-226-2738).

The Sixth Congress of the European College of Neuropsychopharmacology

This congress will take place in Budapest, Hungary, October 10–14, 1993 For information contact Peter Gaszner, Huvosvolgyi út 116, Budapest, H-1021, Hungary *or* Congress Bureau MOTESZ, Ms Krisztina Szigedi (Tel· 361 251-7999, 163-0037, 163-3899; Fax. 361 183-7918).

Volvo Award for CNS Injury Research, 1993 by the Neurotraumatology Committee of the World Federation of Neurosurgical Societies

In order to encourage research aimed at injury prevention analysis and mitigation of central nervous system injuries, the Volvo Company of Göteborg, Sweden has sponsored an award for 1993 of US$5000.

Papers entering the contest must contain **original** material, not previously submitted for publication Multiple authors is acceptable The manuscripts, in the English language, should be full-length, including original illustration, in a form suitable for submission as an original paper (not postgraduate thesis) to a scientific journal One original and five copies of each paper in full must reach the address below **not later than February 1, 1993**

One of the authors should be prepared to come to Acapulco, Mexico, at his own expense, for the 10th International Congress on Neurological Surgery, October 17–22, 1993 to present the paper and to receive the award

The board of referees will be chaired by Professor Daniel Stålhammar and will contain members chosen by the Neurotraumatology Committee of the World Federations of Neurosurgical Societies. Please direct all correspondence to Daniel Stålhammar, Division of Neurosurgery, Sahlgren Hospital, S-413 45 Goteborg, Sweden

THE BEST IN NEUROSCIENCE

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Neuronal cell lines provide large amounts of homogenous cells for biochemical and molecular studies of nervous system function. This book details methods for culturing and using these powerful tools.

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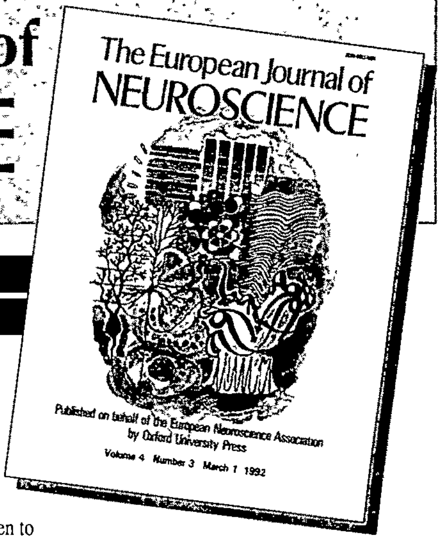
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